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Mitochondrial Long Chain Fatty Acid Oxidation Related Enzyme Changes in Different Preeclampsia-Like Mouse Models

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Abstract

Background: Long-chain Free Fatty Acids (FFA) and long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) are related to some preeclampsia. We studied the relationship between LCHAD, carnitine palmitoyltransferase (CPT) I and II and FFA in different preeclampsia-like models.

Methods: Classic preeclampsia models with Nw-nitro-L-arginine-methyl ester (L-NA) or Lipopolysaccharide (LPS) injection, antiphospholipid syndrome model with β2 glycoprotein I (β2GPI) injection, and ApoC3 mice with abnormal fatty acid metabolism (ApoC3+NS and ApoC3+L-NA) were used. Controls were saline injected. At days 14 and 18 of pregnancy, serum FFA levels were compared and placental and hepatic LCHAD, CPT I and II mRNA and protein were detected using real-time quantitative PCR and western blot.

Results: Serum FFA levels increased significantly except in LPS, compared to controls ($P<0.05$). CPT I mRNA and protein increased significantly in the liver and placenta of L-NA and β2GPI and liver of ApoC3+L-NA ($P<0.05$). CPT II mRNA and protein increased significantly in the liver and placenta of L-NA and the liver of ApoC3+L-NA and β2GPI ($P<0.05$). LCHAD mRNA and protein increased significantly in the liver and placenta of ApoC3+NS, ApoC3+L-NA and β2GPI, and decreased significantly in L-NA ($P<0.05$). Serum FFA levels positively correlated with liver CPT I mRNA and protein in all groups ($P<0.05$) and negatively correlated with liver LCHAD mRNA and protein expression in all groups except in β2GPI group ($P<0.05$).

Conclusions: Changes and correlations in FFA levels and LCHAD, CPT I and II were different according to preeclampsia-like models. β-oxidation disorders may occur at different stages.

Keywords: Pre-Eclampsia; Free fatty acids; Carnitine Palmitoyl transferase; Long-Chain 3-hydroxyacyl-CoA dehydrogenase

Introduction

Preeclampsia is a serious obstetric complication and a major cause of maternal and perinatal mortality and its pathogenesis is still unclear. We and other researchers have proven that long-chain Fatty Acid Oxidation (FAO) is associated with some forms of preeclampsia.

Most fatty acids in the human body are long-chain fatty acids. The β-oxidation of fatty acids occurs in the mitochondria. After activation of long-chain fatty acids, they need assistance from carnitine as a carrier and catalysis of carnitine palmitoyltransferase I (CPT I) in the outer mitochondrial membrane and carnitine palmitoyltransferase II (CPT II) in the inner mitochondrial membrane to enter into the mitochondria [1]. Long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) is a component of the mitochondrial trifunctional protein and is capable of catalyzing the third step of β-oxidation [2]. So CPT I, CPT II and LCHAD are key enzymes in the long-chain FAO process, and defects or dysfunction of which can result in disorders in long-chain fatty acid transport into the mitochondria or oxidation, further resulting in inefficient energy release from FAO and affecting cell function.

In a normal pregnancy, prolactin and estrogen increase the activity of hormone sensitive lipase, resulting in increased fat mobilization, increased maternal plasma Free Fatty Acids (FFA) and increased hepatic uptake of fatty acids. In the past, FAO was generally considered unimportant in placental and fetal development; however, a recent study found that this was not the case. Shekhawat et al. found that LCHAD and six other fatty acid β-oxidation enzymes were abundantly expressed in the placenta, indicating the long-chain FAO played an important role in placental development and energy supply [3]. Oey et al. found Very Long Chain Acyl-Coenzyme a Dehydrogenase (VLCAD) and LCHAD mRNA highly expressed during different stages in the embryo and fetal heart, liver and other tissues, and there were strong VLCAD, LCHAD and CPT II enzyme activities [4]. So long-chain FAO exists not only in the placenta, but also in embryonic tissues, and plays an important role in early development of the fetus. Thus, in a normal pregnancy placental and fetal development also requires FAO as an energy supply.

Previous studies have found that LCHAD was associated with some forms of preeclampsia. Bartha and colleagues reported that LCHAD mRNA expression decreased in the placenta of preeclampsia patients compared with controls, and that the FAO ability of the placenta in preeclampsia patients was reduced [5]. Robinson et al. found that the plasma of preeclampsia patients could lead to lipid droplet aggregation in cultured human umbilical vein endothelial cells and decreased mitochondrial dehydrogenase activity [6]. In our previous studies we also found LCHAD expression decreased in the placenta with early-onset severe preeclampsia, but there was no significant difference between late-onset preeclampsia and the control groups [7]. In animal experiments, we found that LCHAD expression was significantly reduced in the placenta of preeclampsia-like mice during the early and

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middle stages of pregnancy [8]. In our in vitro studies using serum from preeclampsia patients for placental trophoblast cell culture, we found that LCHAD gene and protein expression decreased more obviously with serum from early onset severe preeclampsia and hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome than with serum from the late-onset severe preeclampsia group [9]. When trophoblast cells were cultured with FFAs of different chain lengths, LC-FFA showed similar effects on the LCHAD gene and protein expression to serum from preeclampsia patients [10]. These studies show that reduced expression of LCHAD and FAO disorders play an important role in the occurrence and development of some forms of preeclampsia.

Although studies have shown that long-chain FAO was associated with the pathogenesis of preeclampsia, not all preeclampsia patients have FAO disorders. No changes in Triglyceride (TG) levels were found in a mouse model of reduced uterine perfusion pressure [11]. And our previous studies have shown no significant difference in LCHAD protein expression between late-onset preeclampsia and controls [8]. So we speculate that preeclampsia that is induced by different pathogenic factors or different onset times might exhibit different FAO disorders. In this study, we used two classic preeclampsia-like models and two models associated with preeclampsia occurrence-Antiphospholipid Syndrome (APS) and lipid metabolism disorder models to explore the different aspects of long-chain FFA oxidation, and to explore methods for intervention in the prevention of preeclampsia.

Materials and Methods

Establishment and identification of animal models

The animal experiments were approved by the Animal Care Committee and Medical Ethics Committee of Peking University. C57BL/6J mice were obtained from the Department of Laboratory Animal Science, Peking University, and C57BL/6J mice with transgenic over expression of apolipoprotein C3 (ApoC3) were supported by the Institute of Cardiovascular Sciences, Peking University Health Science Center. We housed 8- to 10-week-old virgin female and 10 to 14 week-old male mice under controlled conditions and fed them standard mouse chow with water available ad libitum. The mice were mated at a ratio of 2:1 females to males and females were inspected daily for vaginal plugs, and then designated as day 1 of pregnancy.

Mice were randomly divided into control, ApoC3+NS, ApoC3+L-NA, L-NA, LPS and β2GPI groups. Except for the β2GPI group, the other groups were subdivided into early-gestation (Early) and mid-gestation (Mid) subgroups according to injection time (n=10 per group). Transgenic mice in the ApoC3+L-NA group and wild-type mice in the L-NA group received a daily subcutaneous injection of Nw-nitro-L-arginine-methyl ester (L-NAME) (Sigma, USA), 50 mg/kg/d [12,13], from day 7 or 11 of pregnancy. For the LPS mice, wild-type mice received a single injection with an ultra-low dose of Lipopolysaccharide (LPS) (1 μg/kg body weight, Sigma) [14,15]. The β2GPI mice were subcutaneously injected weekly with complete Freund's adjuvant-dissolved human β2 glycoprotein I (β2GPI) (25 μg per mouse, Sigma) in the back 3 weeks before mating and incomplete Freund's adjuvant-dissolved β2GPI 2 weeks and 1 week before mating [16]. Wild-type mice in the control group and transgenic mice in the ApoC3+NS group were injected daily with physiological saline from day 7 or 11 of pregnancy.

From day 2 of gestation, a CODA non-invasive tail-cuff acquisition system (Kent Scientific Corp., USA) was used to measure blood pressure every 2 days. The mice were placed in standard metabolism cages on day 17 of pregnancy and 24-hr urine was collected. The detection of urinary protein involved a protein assay kit (Bio-Rad, USA).

Sample collection

All mice were anesthetized with 10% chloral hydrate (3 ml/kg) on day 14 (14D group) and day 18 (18D group) of pregnancy. Blood samples, taken immediately from the retro-orbital plexus, were centrifuged and serum was collected. Cesarean section was performed, and the placenta and liver tissues were collected and frozen at -80°C for mRNA and protein detection. Finally the mice were terminated by cervical dislocation.

Serum FFA assay

Serum FFA levels were assayed with a chemical analysis kit (Wako Chemicals, Japan) according to the instructions.

Quantitative real-time PCR

TRIzol reagent (Sigma, USA) was used to extract total RNA from the liver and placenta. Total RNA, 1 μg, was reverse-transcribed to cDNA by use of the Revert Aid First Strand cDNA Synthesis Kit (Thermo, USA). The real-time quantitative PCR reaction system involved SYBR Select Master Mix reagent (Invitrogen Life Technologies, USA) and PCR amplification involved a 7500 Real-Time PCR System (Life Technology, USA). Primer synthesis was completed by Sangon Biotech (Shanghai) with the primer sequences for CPT I, forward, 5'-TGGCATCATCACTGGTGTGTT-3', and reverse, 5'-GTCTAGGGTCCGATTGATCTTTG-3'; CPT II, forward, 5'-CAGCACAGCATCGTACCCA-3', and reverse, 5'-GTTGGCCCAGATTTCGTTCA-3'; LCHAD, forward, 5'-TGCATTTGCCGCAGCTTTAC-3', and reverse, 5'-TCCCAATGCCGTTCTCAAAAT-3'; and GAPDH (as an internal control) forward, 5'-TGATGACATCAAGAAGGTGGTGAAG-3', and reverse, 5'-TCCTTGGAGGCCATGTAGGCCAT-3'. PCR conditions were 94°C for 2 min; 55-60°C for 30 s and 72°C for 1 min, 40 cycles.

Western blotting analysis

Protein was extracted from liver and placenta tissues by use of RIPA lysis buffer (cwbiotech, China) with prior addition of protease inhibitors (Pierce, USA). An equal amount of protein sample was used for electrophoresis in 8% or 10% polyacrylamide gels and transferred onto a 0.45 μm polyvinylidene difluoride (PVDF) membrane (Millipore, USA), which was blocked with 5% milk (BD, USA) at room temperature for 1 hr, then incubated with primary antibodies rabbit anti-mouse LCHAD (Abcam, UK; 1:500), rabbit anti-mouse CPT I (Abcam, UK; 1:500), CPT II (Abcam, UK; 1:1000) and rabbit anti-mouse β-actin (Cell Signaling, USA; 1:1000) at 4°C overnight. Membranes were washed at room temperature for 5 min×5 times and then horseradish peroxidase-conjugated secondary antibody was added (1:10000, Thermo, USA) for incubation at room temperature for 1 hr and then washed again for 5 min×5 times. The KODAK gel logic 4000MM PRO imaging system (Kodak, USA) was used for scanning and detection of bands. The relative expression of the target protein compared to β-actin was calculated.

Statistical analysis

SPSS 20.0 (IBM, USA) was used for data analysis. Quantitative data are expressed as mean ± SD. One-way ANOVA followed by

Student-Newman-Keuls or Games-Howell test was used for comparing multiple groups. Pearson correlational analysis was used. P< 0.05 was considered statistically significant.

Results

Confirmation of PE models

The ApoC3+NS group showed only gestational hypertension symptoms, but after injection with L-NAME, blood pressure and urine protein in the ApoC3+L-NA group was significantly higher (P<0.05) than in the ApoC3+NS group, showing preeclampsia-like symptoms. The other groups exhibited preeclampsia-like symptoms including hypertension and proteinuria after administration. Mean arterial pressure (MAP) in the β2GPI group from day 2 of pregnancy, and in the ApoC3+L-NA, L-NA and LPS groups from the second day after administration was significantly higher than the control group (P<0.05), and MAP increased along with gestational time. MAP in the early subgroups of the ApoC3+L-NA, L-NA and LPS groups was significantly higher (P<0.05) than in the corresponding mid subgroups (Figure 1A-1D). There were no significant differences in urine protein levels between the ApoC3+NS group and the control group, while urine protein levels in the other groups were significantly higher than in the

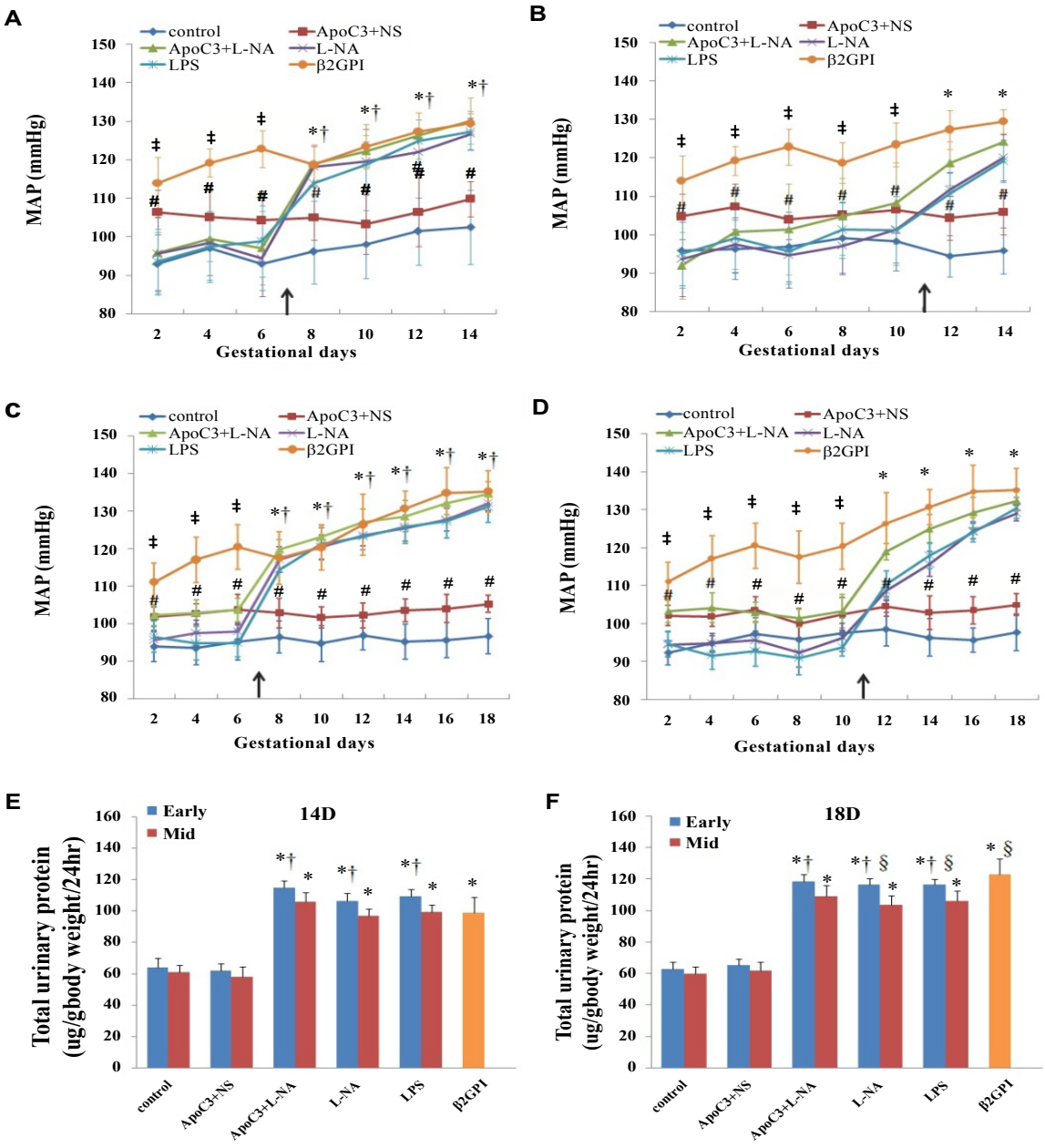

Figure 1: Mean arterial blood pressure (MAP) (A-D) and 24-hour urinary protein levels (E-F) in all groups. The arrow indicates time point of injection except β2GPI group. *p<0.05 compared with control and ApoC3+NS. #P<0.05 compared with control. ‡p<0.05 compared with all other groups. †P<0.05, compared with corresponding Mid group. §P<0.05, compared with corresponding 14D group. Data are mean ± SD, n=10 per group.

control group (P<0.05). Urine protein levels in the early subgroup of the ApoC3+L-NA, L-NA and LPS groups increased significantly compared with the corresponding mid subgroups (P<0.05). When 18D groups compared with the corresponding 14D groups, urinary protein levels in the L-NA, LPS and β2GPI groups were significantly increased (P<0.05) (Figure 1E and 1F).

FFA and LCHAD changes in different preeclampsia -like mouse models

In both the 14D and 18D groups, except for the LPS group, serum FFA levels increased significantly compared to the control group (P<0.05). FFA levels in the early subgroup of the 18D ApoC3+L-NA group were significantly higher than corresponding mid subgroup (P<0.05). FFA levels in the 18D early ApoC3+NS and ApoC3+L-NA groups increased significantly compared with the corresponding 14D groups (P<0.05) (Figure 2A and 2B). Compared with the corresponding control group, LCHAD mRNA and protein expression in the liver and placenta significantly increased in the ApoC3+NS, ApoC3+L-NA and β2GPI groups (P<0.05) and significantly decreased in the L-NA group (P<0.05), while no significant difference was found in the LPS group. There was no significant difference in LCHAD mRNA and protein expression between early subgroups and corresponding mid subgroups. There was also no significant difference between 18D and 14D groups

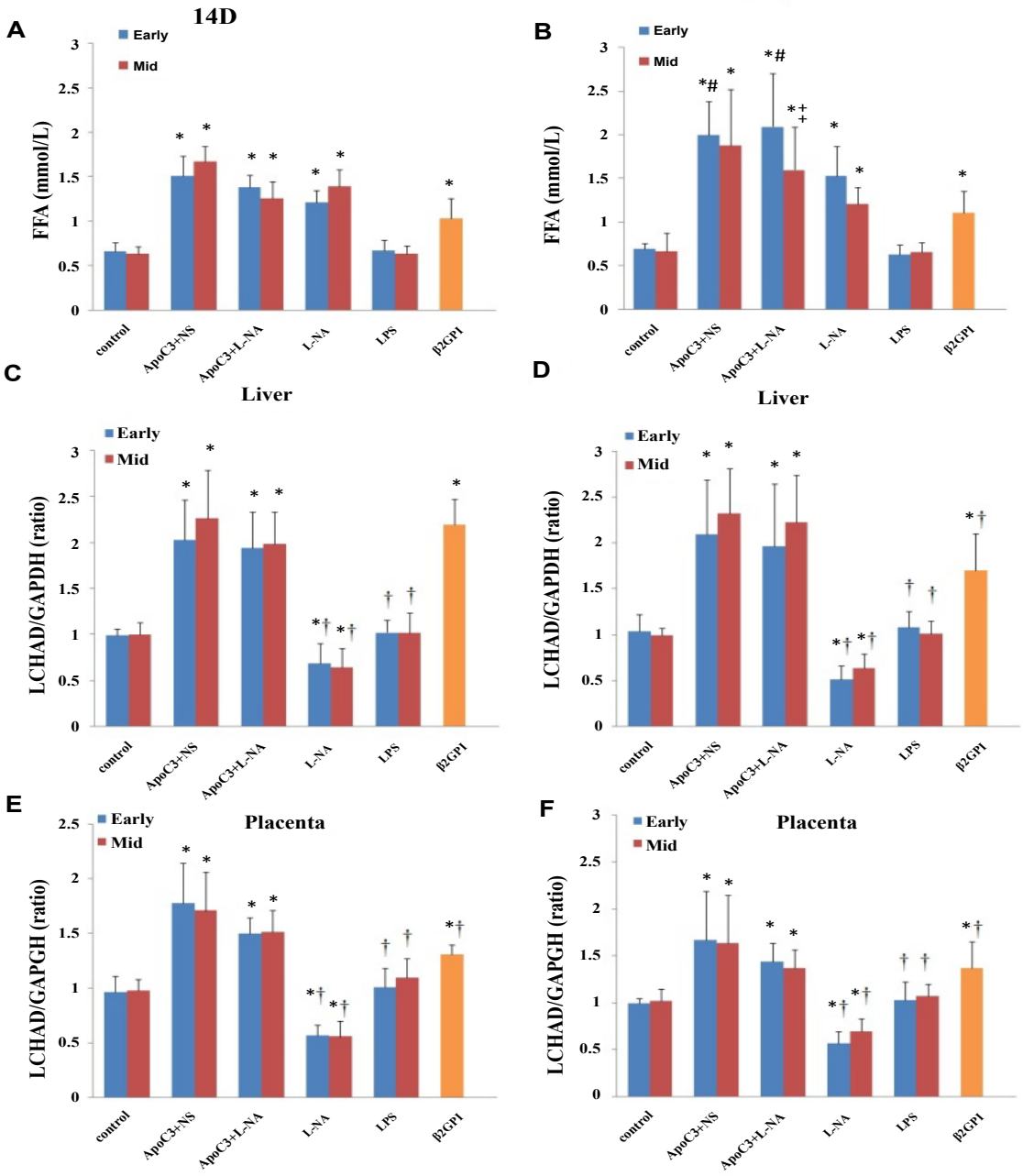

Figure 2: Free fatty acid (FFA) levels (A,B) and long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) mRNA expression in maternal liver and placenta (C,F) in all groups.*p<0.05 compared with control. ‡P<0.05, compared with corresponding Early group. #P<0.05 compared with corresponding 14D group. †P<0.05, compared with ApoC3+NS and ApoC3+L-NA group. Data are mean ± SD, n=10 per group.

(Figures 2C-F and 3). LCHAD protein expression in the liver of the 14D early ApoC3+L-NA group was significantly decreased compared to the corresponding ApoC3+NS group (P<0.05), and significantly increased compared with the other groups (P<0.05) (Figure 3A and 3B). LCHAD protein expression in the placenta of the ApoC3+L-NA group was significantly lower than in the ApoC3+NS group (P<0.05) (Figure 3C and 3D).

CPT I and II mRNA expression in the liver and placenta of different preeclampsia-like mouse models

Compared with the corresponding control group, CPT I mRNA expression significantly increased in the liver of the ApoC3+L-NA, L-NA and β2GPI groups (P<0.05), while in the ApoC3+NS and LPS groups there were no significant differences (Figure 4A and 4B). CPT I mRNA expression in the placenta of the L-NA and β2GPI groups was significantly increased (P<0.05), while there was no significant difference in the other groups (Figure 4C and 4D). CPT I mRNA expression increased significantly in the early subgroup of the 18D L-NA group compared with the corresponding mid subgroup (P<0.05), while there was no significant difference in the early subgroups and mid subgroups of the other groups. In the 18D groups compared with the corresponding 14D groups, CPT I mRNA expression in liver of early ApoC3+L-NA and β2GPI groups was significantly increased (P<0.05) (Figure 4A and 4B).

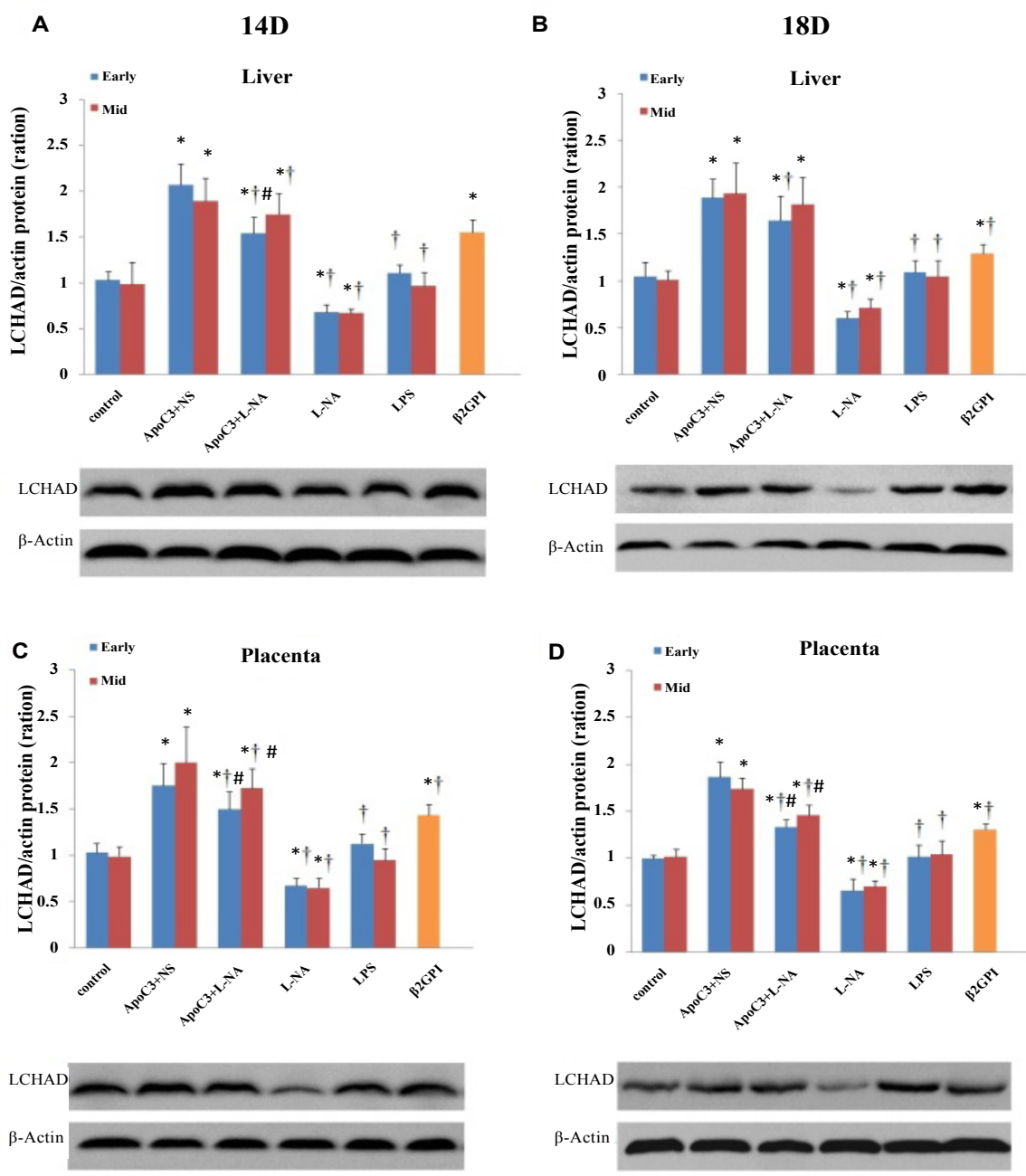

Figure 3: Long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) protein expression in maternal liver and placenta in all groups.*p<0.05, compared with control. †P<0.05, compared with ApoC3+NS and ApoC3+L-NA group. #P<0.05 compared with corresponding ApoC3+NS group. Data are mean ± SD, n=10 per group.

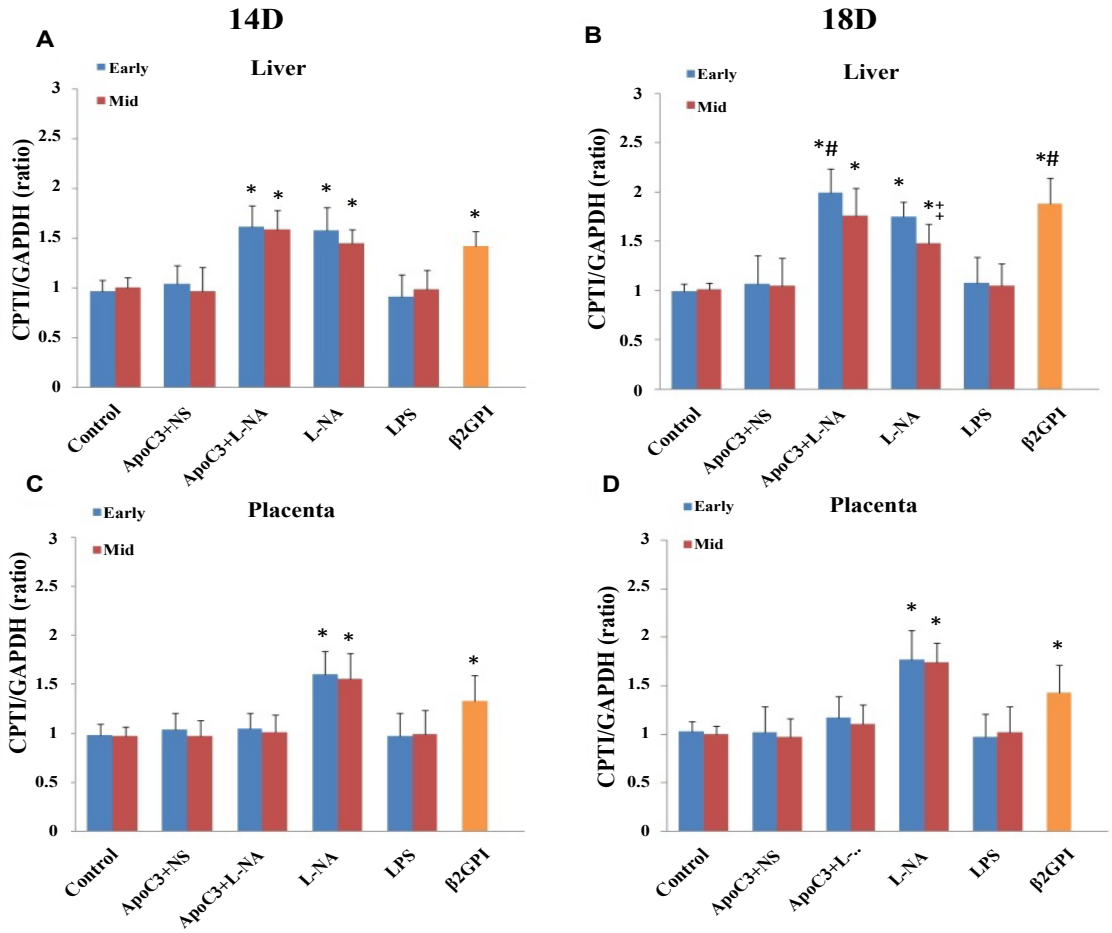

Figure 4: Carnitine palmitoyltransferase I (CPTI) mRNA expression in maternal liver and placenta in all groups.*p<0.05, compared with control. ‡P<0.05, compared with corresponding Early group. #P<0.05 compared with corresponding14D group. Data are mean ± SD, n=10 per group.

Compared with the corresponding control group, CPT II mRNA expression significantly increased in the liver of the ApoC3+L-NA, L-NA and β2GPI groups (P<0.05), while in the ApoC3+NS and LPS groups there were no significant differences (Figure 5A and 5B). CPT II mRNA expression in the placenta of the L-NA group was significantly increased (P<0.05), while there was no significant difference in the other groups (Figure 5C and D). There was no significant difference in CPT II mRNA expression between early subgroups and the corresponding mid subgroups, and also between 18D groups and the corresponding 14D groups.

CPT I and II protein expression in the liver and placenta of different preeclampsia-like mouse models

Compared with the corresponding control group, CPT I protein expression significantly increased in the liver of the ApoC3+L-NA, L-NA and β2GPI groups (P<0.05), while in the ApoC3+NS and LPS groups there were no significant differences (Figure 6A and 6B). CPT I protein expression in the placenta of the L-NA and β2GPI groups was significantly increased (P<0.05), while there was no significant difference in the other groups (Figure 6C and 6D).

Compared with the corresponding control group, CPT II protein expression significantly increased in the liver of the ApoC3+L-NA, L-NA and β2GPI groups (P<0.05), while in the ApoC3+NS and LPS groups there were no significant differences (Figure 7A and 7B). CPT II protein expression in the placenta of the L-NA group was significantly increased (P<0.05), while there was no significant difference in the other groups (Figure 7C and 7D).

There was no significant difference in CPT I and CPT II protein expression between early subgroups and the corresponding mid subgroups, and also between 18D groups and the corresponding 14D groups.

Correlation analysis

Serum FFA levels in the ApoC3+NS, ApoC3+L-NA and L-NA groups showed a significant negative correlation with LCHAD mRNA and protein expression in liver (P<0.05), while in the β2GPI group they showed a significant positive correlation (P<0.05). Serum FFA levels in each group showed no significant correlation with LCHAD mRNA and protein expression in the placenta. Serum FFA levels in ApoC3+L-NA, L-NA and β2GPI groups were significantly positively correlated with CPT I mRNA and protein expression in the liver (P<0.05), but in all groups they were not significantly associated with CPT I mRNA and protein expression in the placenta. Serum FFA levels in all groups were not significantly correlated with CPT II mRNA and protein expression in either the liver or placenta.

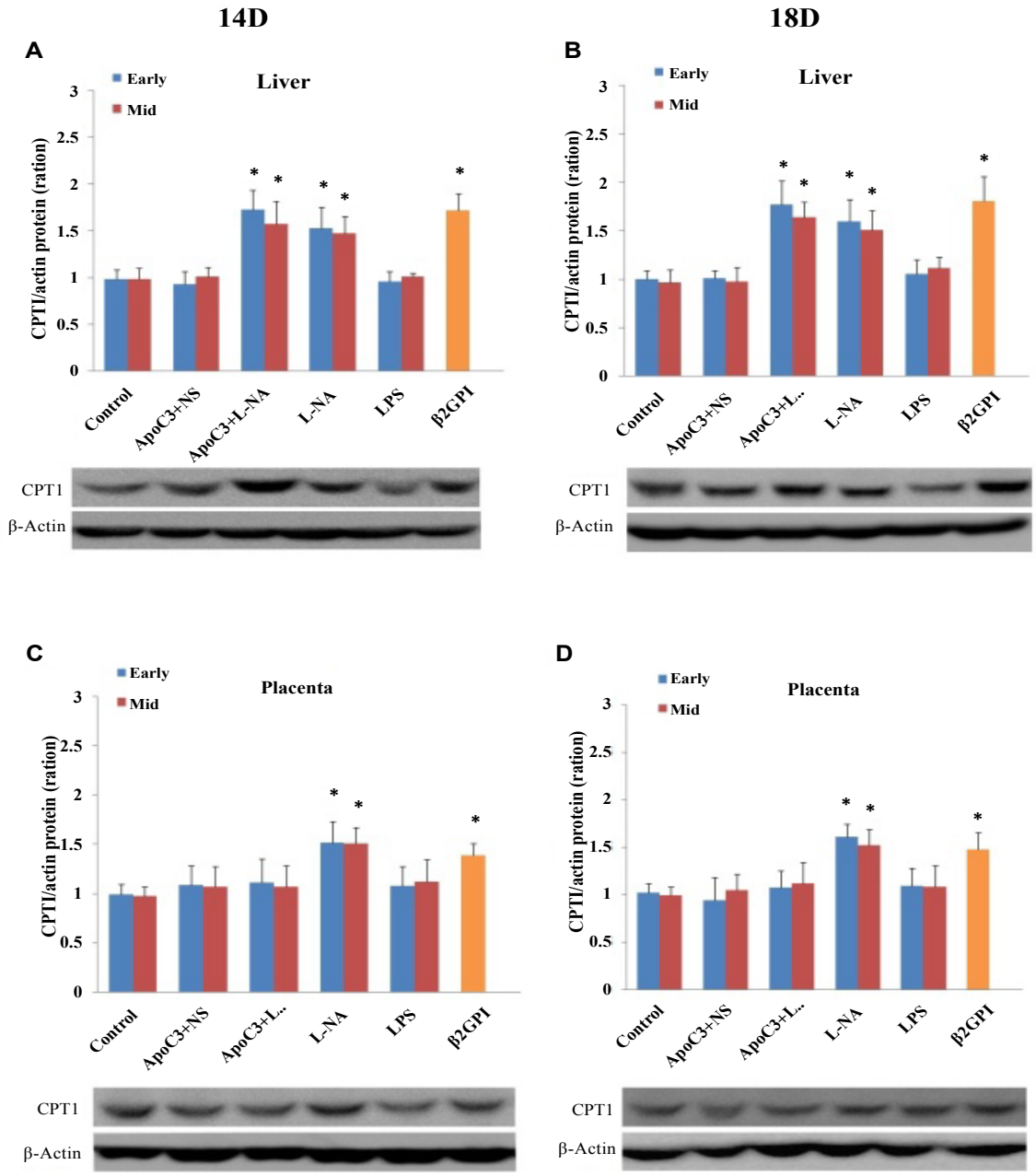

Figure 5: Carnitine palmitoyltransferase II (CPTII) mRNA expression in maternal liver and placenta in all groups.*p<0.05, compared with control. †P<0.05, compared with ApoC3+L-NAgroup. Data are mean ± SD, n=10 per group.

Discussion

Preeclampsia is a multifactorial disease, and there are many different theories about its pathogenesis [17]. Many methods are used to establish preeclampsia-like animal models to investigate the etiology, pathogenesis and treatment of preeclampsia, including uterine artery ischemia, angiogenesis disorders, nitric oxide synthase inhibitors, endotoxin injection, inflammatory factor injections and genetic defects [18]. Although these preeclampsia-like animal models can explain the relationship between one factor and the etiology or pathogenesis of preeclampsia, they lack relevance in the study of multiple pathogenic factors associated with preeclampsia. Our previous studies found both LC-FFA and the serum of preeclampsia patients could lead to decreased LCHAD gene and protein expression in cultured trophoblasts [9,10]. In the present study, we established four different preeclampsia-like animal models as a multi-factor research platform, to explore the changes and effects of long-chain FAO disorders in different factor-induced and different onset time preeclampsia models.

Previous studies have demonstrated that LCHAD deficiencies are related to some forms of preeclampsia and gestational idiopathic liver damage such as HELLP and AFLP (Acute Fatty Liver of Pregnancy), but recent studies have found LCHAD expression is decreased in the placenta of some preeclampsia patients without LCHAD deficiency.

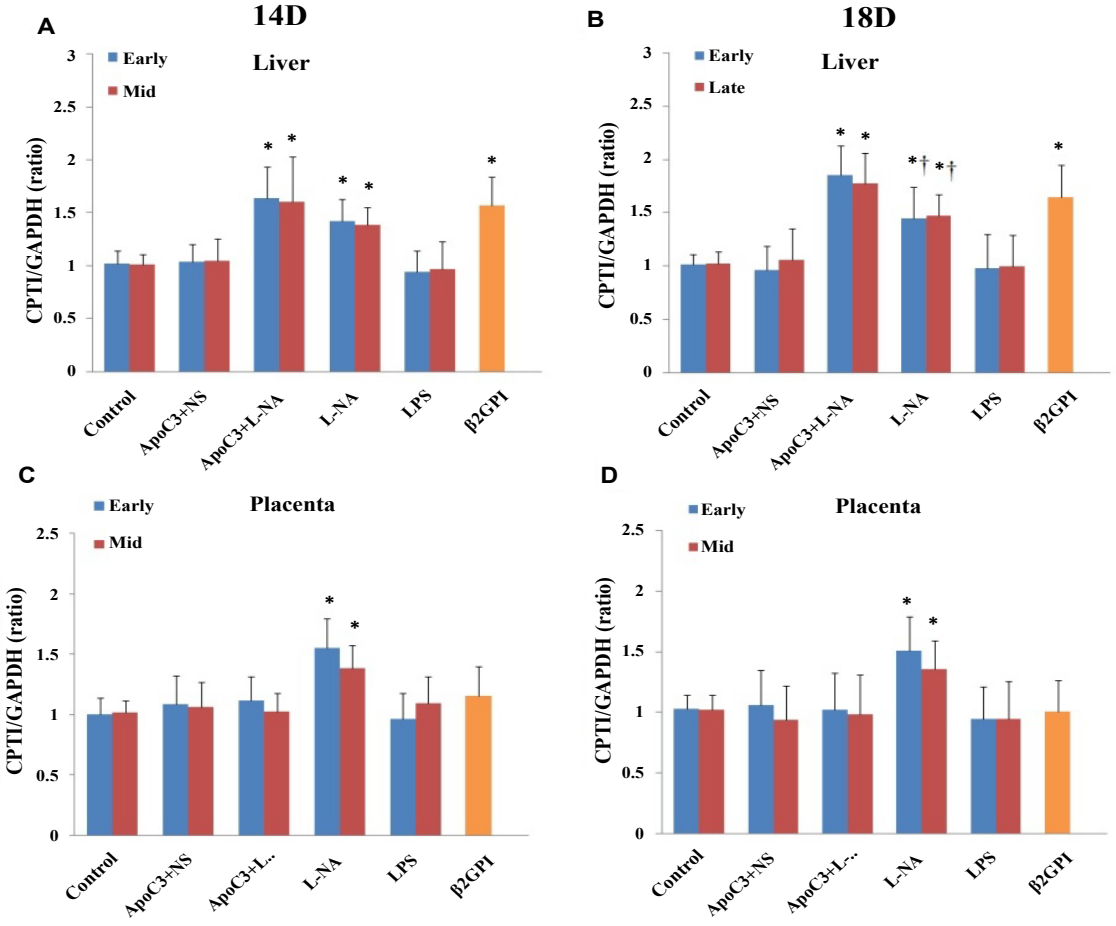

Figure 6: Carnitine palmitoyltransferase I (CPTI) protein expression in maternal liver and placenta in all groups.*p<0.05, compared with control. Data are mean ± SD, n=10 per group.

There are few reports about LCHAD changes in preeclampsia induced by different factors. In this study we found different LCHAD changes in different preeclampsia-like mouse models. LCHAD mRNA and protein expression in the ApoC3+NS group was significantly higher than the control group, indicating that high expression of ApoC3 caused high triglyceride levels resulting in enhanced FAO. Expression of LCHAD mRNA and protein decreased in the L-NA group, which indicated that L-NAME possibly increased lipolysis, reduced the activity of fatty acid oxidase leading to FAO disorders, thereby increasing the accumulation of intermediate products, leading to intracellular lipid accumulation, and finally lipotoxicity, or is associated with oxidative stress and endothelial damage caused the occurrence of preeclampsia. LCHAD mRNA and protein expression in the LPS group showed no significant difference to the control group, indicating that LPS might participate in the development of preeclampsia by other inflammatory pathways. LCHAD mRNA and protein expression were significantly higher in β2GPI group compared with the control group, indicating LCHAD might also affect FAO through other mechanisms.

Abnormal lipid metabolism as a maternal underlying disease may be one factor in the pathogenesis of preeclampsia [19]. Clinical studies have found that patients with preeclampsia, especially early-onset preeclampsia, had existing abnormal lipid metabolism, involving FFAs [20], triglycerides [21,22], cholesterol [23], low-density lipoprotein [24] and phospholipids [25]. FFAs are not only used as mitochondrial substrates for FAO to supply energy, they also have a role in oxidative stress and inflammatory signalling pathways; this role has had much attention during research into the pathogenesis of preeclampsia. In this study we found that FFA levels in all groups except in the LPS group were significantly higher than in the control group. Serum FFA levels in the ApoC3+NS, ApoC3+L-NA and L-NA groups were significantly negatively correlated with LCHAD mRNA and protein expression in the liver, and significantly positively correlated in the β2GPI group. Serum FFA levels in each group were not significantly correlated with LCHAD mRNA and protein expression in the placenta. These results indicated that elevated serum FFA levels in the ApoC3+NS, ApoC3+L-NA and L-NA groups might be due to decreased expression of liver LCHAD. The elevated serum FFA levels in the β2GPI group might be caused by other factors. Between serum FFA levels and LCHAD mRNA and protein expression in the placenta there was no significant correlation, possibly because the liver is the main organ of FAO and the main cause of the overall changes in serum FFA. Studies have found that during pregnancy LCHAD, short-chain 3-hydroxyacyl-CoA dehydrogenase (SCHAD), long-chain 3-ketoacyl-CoA thiolase (LKAT) and CPT II activities in the liver were 2-5 times those of the placenta [3].

FFAs entering the mitochondria for β oxidation need transmembrane transport with the assistance of CPT I and II catalysis. Acyl-CoA entering the mitochondria is the major rate-limiting step of

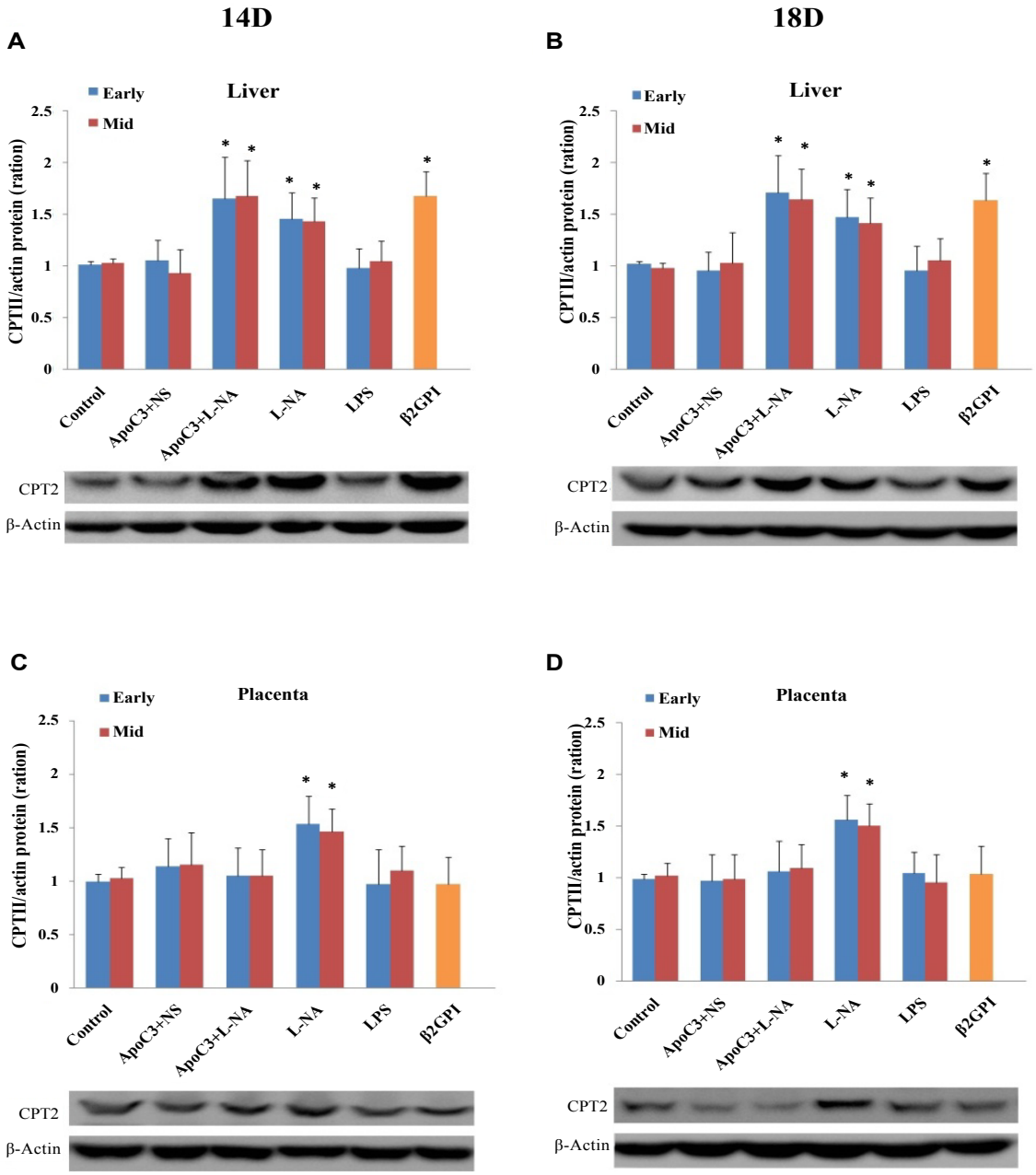

Figure 7: Carnitine palmitoyltransferase II (CPTII) protein expression in maternal liver and placenta in all groups. *p<0.05 compared with control. Data are mean ± SD, n=10 per group.

fatty acid β oxidation, and CPT I is the rate-limiting enzyme. Previous studies have found CPT I or II defects could cause early fetal death [26], indicating that they play an important role in the development of the fetus. So, abnormal CPT I or II expression may cause long-chain FAO and lipid metabolism disorders in preeclampsia. In this study we found that CPT I expression was significantly increased in the liver of the ApoC3+L-NA group and in the liver and placenta of the L-NA and β2GPI groups. Correlation analysis showed that FFA levels were significantly positively correlated with CPT I expression in the liver, but not in the placenta, indicating that increased serum FFA could lead to increased CPT I expression, leading to increased FFA transport into the mitochondria for β oxidation. Increased CPT I expression might be a compensatory behavior. Studies have found that long-chain fatty acids could directly stimulate CPT I gene expression in the liver independent of the peroxisome proliferator-activated receptor alpha pathway [27]. In normal liver, regulation of mitochondrial FAO mainly relies upon CPT I gene expression levels that are altered in response to physiological or pathological stimuli. Starvation, high-fat diet, induced diabetes and mitochondrial proliferation drugs increase CPT I mRNA expression, while CPT II is not significantly changed [28]. In this study we found that CPT II mRNA and protein expression were significantly increased in the liver of the ApoC3+L-NA and β2GPI groups, and in the liver and placenta of the L-NA group. Correlation analysis showed that FFA levels and CPT II mRNA and protein

expression in the liver and placenta had no significant correlation, indicating increased CPT II expression might have nothing to do with the FFA increase in the cytoplasm, and might be associated with the accumulation of mitochondrial metabolites. Studies have found that the CPT I and II catalytic reaction is reversible, when mitochondrial FAO disorders resulted in the accumulation of acyl-CoA, CPT II was capable of catalyzing the acyl-CoA and carnitine to form acyl carnitines and transporting them outside the mitochondria, thereby reducing the accumulation of fatty acid metabolism products in the mitochondria or in the cell [29].

Mice in the ApoC3+NS group had abnormal lipid metabolism. In this study FFA levels and LCHAD mRNA and protein expression were increased in the ApoC3+NS group possibly due to the high expression of ApoC3 resulting in high triglyceride and finally enhanced FAO. CPT I and II mRNA and protein expression in the ApoC3+NS group did not change significantly, probably because elevated FFA only stimulated CPT I and II activity and did not change their expression. However, after the acceptance of adverse factors during pregnancy, altered CPT I and II gene and protein levels in the liver of the ApoC3+L-NA group indicated that L-NA further exacerbated the burden of FAO finally inducing increased CPT I and II gene and protein expression.

L-NAME is an endothelial nitric oxide synthase inhibitor and our previous studies showed that L-NAME injected into C57BL/6J mice could lead to preeclampsia-like symptoms and long-chain FAO disorders [8]. Consistent with our previous studies, LCHAD mRNA and protein expression in the L-NA group decreased. Studies have found that L-NAME can cause elevated serum Total Cholesterol (TC) and TG, and decreased hepatic Carnitine Palmi-Toyltransferase (CPT) activity, resulting in FAO disorders [30]. In the present study reduced LCHAD expression led to a reduced mitochondrial oxidative capacity resulting in the accumulation of mitochondrial fatty acid metabolites. High expression of CPT I and II might transport these metabolites outside the mitochondria to avoid damage to the mitochondria, possibly from compensatory behavior. Increased CPT I expression can result in low levels of carnitine which is a powerful antioxidant in cells [31], and lead to a reduced antioxidant capacity.

FFA levels and FAO related enzymes in the LPS group showed no significant difference compared with the control group, indicating that LPS might participate in the development of preeclampsia by other inflammatory pathways. LPS activates macrophages to produce interleukin 1, interleukin 6 and tumor necrosis factor-alpha (TNF-α) cytokines, which act on endothelial cells and activate nuclear factor kappaB (NF-κB) and other inflammatory pathways, leading to endothelial cell injury and dysfunction, and resulting in preeclampsia-like symptoms. Long-chain FAO disorders may not be the main pathogenesis in this preeclampsia-like model. Clinical and basic studies have found lipid-lowering drugs like statins could prevent the occurrence of some forms of preeclampsia, but this conclusion is still controversial [32]. In this study abnormal lipid metabolism was not found in the LPS models, indicating that there are obviously different lipid metabolism changes in the different preeclampsia-like models, and further illustrating that there is not a single preventive measure to avoid the onset of all forms of preeclampsia, and that it is a multi-factorial disease in humans. Preventive measures against maternal underlying conditions and the corresponding pathogenesis could obtain better results.

In this study, serum FFA levels in the β2GPI group were positively correlated with LCHAD expression, indicating that LCHAD might affect FAO through other mechanisms. Aylor et al. have found that LCHAD plays an important role in Cardiolipin (CL) remodelling [33]. We speculate that increased anti-cardiolipin antibodies and anti-β2GPI antibodies may bind to CL on the mitochondrial membrane, thus affecting mitochondrial function. The body up regulates LCHAD expression in a compensatory manner to synthesize more CL, which leads to a decreased role for LCHAD as a long-chain FAO enzyme, thus resulting in fatty acid β oxidation disorders. High expression levels of CPT I and II in this model may transport these metabolites outside the mitochondria to avoid mitochondrial damage.

It is difficult to obtain liver tissues from patients with preeclampsia in clinical studies. Usually placental tissues can only be collected after delivery, when it is difficult to determine whether physiological and pathological changes in the placenta are the reasons for, or the results of, the occurrence of preeclampsia. Using animal models we obtained specimens at an early gestational stage. In this study, FFA levels and FAO related gene and protein expression changed at 14D, and at 18D compared to 14D, FFA levels in the early ApoC3+NS and ApoC3+L-NA groups significantly increased; CPT I mRNA expression was significantly increased in the liver of the early ApoC3+L-NA group and in the placenta of the mid ApoC3+L-NA group. These results indicated that FAO disorders had emerged in the mid gestational stage, and were aggravated with progression of pregnancy time.

In conclusion, there is a close relationship between FAO disorders and the development of some (but not all) preeclampsia. The pattern and extent of FAO disorders are different in the different preeclampsia-like models, affecting different aspects of long-chain FAO. Long-chain FAO in different factor-induced preeclampsia models, whether produced by oxidative stress or endothelial dysfunction, ultimately causes preeclampsia, but the interaction and impact need further study.

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