Impaired Mitochondrial Fusion, Autophagy, Biogenesis and Dysregulated Lipid Metabolism is associated with Preeclampsia

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Abstract: Preeclampsia(PE) is a pregnancy complication that is diagnosed by the new onset of hypertension and proteinuria. The etiology of PE remains unclear; however, growing evidence indicates that mitochondrial impairment contributes to the pathogenesis. Therefore, we aim to investigate the function of mitochondria in the development of PE. The mitochondrial metabolome in preeclamptic (n = 11) and normal (n = 11) placentas were analyzed using Gas chromatography-mass spectrometry (GC-MS). Student's t-tests and receiver operating characteristic (ROC) curves were conducted to determine which mitochondrial metabolites differed significantly between the two groups. The Pathway Activity Profiling (PAPi) R package was used to predict which metabolic pathways were affected by PE. Western blot analysis was performed to identify the candidate proteins which were associated with mitochondrial repair regulation. GC-MS analysis demonstrated that higher levels of 38 metabolites and lower levels of 2 metabolites were observed in the placenta of patients with severe PE (sPE). Five fatty acids had an area under the ROC curve above 90%. Furthermore, we revealed abnormal regulation of mitochondrial dynamics, autophagy, and biogenesis in sPE. Our discoveries indicate that the compromised lipid metabolism in sPE may result from dysfunctional mitochondria, thus revealing new insights into the etiology of the disease.

Keywords: Preeclampsia; impaired mitochondria; metabolomics; lipid metabolism

1. Introduction

Preeclampsia (PE) complicates up to 3–5% of all pregnancies and is characterized by the new presentation of hypertension and proteinuria after 20 weeks of gestation[1]. Although placental disorders such as oxidative stress, increased inflammation, excessive apoptosis, maternal immune dysfunction, nutritional imbalance have been associated with the development of PE[2-5], the etiology and pathogenesis remain unclear.

It is recognized that one mechanism leading to the development of PE is from shallow trophoblast invasion and failed spiral artery remodeling, which in turn can lead to mitochondrial hypoxia and oxidative stress injury[6]. Mitochondrial dynamics, biogenesis, and autophagy play important roles in maintaining the healthy population of mitochondria[7-9]. Fusion compensates for mitochondrial defects by diluting the damaged mitochondrial respiratory contents and thereby preventing their elimination, while fission promotes the division of an impaired daughter unit, which will then be recognized by mitophagy. Mitophagy is a subtype of macroautophagy, where damaged mitochondria are sequestered in autophagosomes and subsequently digested[10-13]. Mitochondria biogenesis consists of mitochondrial

DNA replication, protein synthesis, and importation. Under certain stresses, peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) and Sirtuin3 (SIRT3) can act as master regulators of mitochondrial DNA replication to meet the energy demands of the cell and counteract cell destruction[14-17].

Although Adenosine Triphosphate (ATP) production is the primary function of mitochondria, numerous studies have demonstrated relationships between dysfunctional mitochondria and human conditions such as Parkinson's disease, Alzheimer's disease, diabetes mellitus, and cardiomyopathies[10]. There is increasing evidence to suggest that mitochondrial dysfunction may exacerbate the development of PE. As the main source of reactive oxygen species (ROS) production, placental mitochondrial disorders have been implicated in the increased oxidative stress observed in PE[18]. In addition, compromised antioxidant protection and higher ROS production were observed in the placental mitochondria from women diagnosed with PE[19, 20]. ROS derived from mitochondria have been implicated in the dysfunction of endothelial cells and antioxidants targeted at reducing mitochondrial ROS stress have been proposed as potential therapeutic candidates for PE[21]. Therefore, the aim of this research was to investigate whether placental mitochondrial dysfunction plays an important role in the pathogenesis of PE.

In this study, we provide evidence that the dysfunction of the mitochondria in severe PE (sPE) is reflected in the placental mitochondrial metabolome. Additionally, we demonstrate that the altered lipid metabolism in sPE may result from the disturbances of mitochondrial fusion, autophagy, and biogenesis and thus significantly attenuate the ability of mitochondria to synthesize ATP. These outcomes offer new understandings as to the pathophysiological mechanisms of PE.

2. Materials and Methods

This investigation conforms with the principles outlined in the Declaration of Helsinki. The study was approved by the Research Ethics Committee of the First Affiliated Hospital of Chongqing Medical University, China. Informed consent was obtained from each participant.

2.1 Patient Characteristics

Pregnant women with sPE (n = 11) and healthy pregnancies (n = 11) were included in this study. Clinical data for all participants are shown in **Table 1.** All babies were delivered via cesarean section and placental tissue was obtained and transferred immediately to the Department of Gynecology and Obstetrics in The First Affiliated Hospital of Chongqing Medical University. The definition of sPE was in accordance with the guidelines of the American College of Obstetrics and Gynecology[22]. sPE was defined as having at least one of the following criteria: systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure ≥ 110 mmHg and/or random urine specimens collected at least 4 hours apart ≥ 3+. Patients with chronic medical disorders such as gestational diabetes mellitus, cardiovascular disease, collagen disorder, chronic renal disease, chronic hypertension, and metabolic diseases were excluded, as well as multiple pregnancies. Women with a healthy pregnancy were used as controls, and were selected so that their maternal age, BMI at delivery, gestational age, and parity were matched with women having sPE. No women in this study reported smoking during pregnancy.

Table 1. Clinical characteristics of study populations

Category	sPE (n=11)	Normal(n=11)	*P* - value
Age (years)	30.0±3.9	31.0±4.3	0.571[a]
BMI at delivery (kg/m2)	31.0±.2.4	28.9±3.3	0.061[b]
Gestational age at delivery (weeks)	37.1±1.4	37.7±0.6	0.716[b]

Parity	1.5±0.8	1.5 ±0.5	1.000[b]
Systolic blood pressure (mmHg)	169.3±16.8	113.5±6.2	<0.0001[b]
Diastolic blood pressure (mmHg)	106.1±8.6	68.2 ±3.9	<0.0001[b]
Proteinuria	2.2+ ±1.4+	0±0	<0.0001[b]
Placental weight(g)	2681.8 ±266.8	3524.5 ±241.9	<0.0001[b]
Neonatal birth weight (g)	475.5±76.7	554.5±43.9	<0.05[b]
Ratio of the newborns(male/female)	6/5	4/7	0.669[C]

sPE: Severe Preeclampsia; [a]Student T-test; [b]Mann-Whitney test; [C]Chi-squared test

2.2 Placental Tissue Collection and Isolation of Mitochondria

Fresh placental tissue was obtained immediately after the cesarean section: five pieces from the maternal side of the placental villous tissue was obtained randomly, avoiding the calcification region, and were kept in cold phosphate buffer saline (PBS) with an ice-bag. The samples were then transferred immediately to the Department of Gynecology and Obstetrics in The First Affiliated Hospital of Chongqing Medical University. Tissues were washed with cold PBS 3 times to eliminate blood. Portions of the fresh placental tissue were used for the isolation of mitochondria, performed following the manufacturer's instructions (Beyotime, Beijing), while the remaining portions were stored in liquid nitrogen for ATP and western blot analysis. In brief, placenta was washed with cold PBS 3 times, then 200 mg of tissue was homogenized in 2ml of mitochondrial isolation solution; the resulting homogenate was centrifuged at 1,000 g for 5 min, and the supernatant was then transferred into a new tube and centrifuged at 3,500 g for 10 mins. Mitochondria were isolated as the precipitant and stored at −80°C.

2.3 Western Blotting

Proteins were extracted from frozen placental tissues with RIPA lysis buffer (Beyotime, China). Protein concentration was measured using a BCA Protein Assay Kit (Beyotime, China), according to the manufacturer's instructions. Western blotting was performed based on the technique established in our laboratory[23]. Protein samples were loaded in SDS-polyacrylamide gels, resolved by electrophoresis and transferred to polyvinylidene difluoride membranes (Millipore, USA). The levels of proteins were quantified by a ChemiDoc™ XRS+ (Bio-Rad, USA). β-actin was used as loading control. Western blotting antibodies for COX4I1,LDH, and GRP78 were used to confirm the purity of isolated mitochondria (Figure S1). The antibodies used were anti-BNIP3 (1:1000, Abcam, Catalog#: ab109362), LDH (1:1000, Abcam, Catalog#: ab52488) , PGC-1α (1:1000, Abcam, Catalog#: ab54481), GRP78(1:1000, Abcam, Catalog#: ab21685) , SIRT3 (1:500, Proteintech, Catalog#: 10099-1-AP), MFN2 (1:500, Proteintech, Catalog#: 12186-1-AP), COX4I1(1:500, Proteintech, Catalog#: 11242-1-AP), MFN1 (1:500, Santa Cruz, Catalog#: sc-166644), OPA1 (1:500, Santa Cruz, Catalog#: sc-393296), DRP1 (1:500, Santa Cruz, Catalog#: sc-271583), Fis1 (1:500, Santa Cruz, Catalog#: sc-376447), and β-actin (1: 2000, ZSGB-BIO, Catalog#: TA-09).

2.4 Quantification of ATP Levels

Measurement of ATP levels was performed with an ATP assay kit, according to the manufacturer's instructions (Beyotime, China). The kit is based on a luciferase-luciferin reaction assay. 20 mg of each frozen placenta was homogenated by the lysis reagent, centrifuged at 12,000 g for 5 min, and then 100 µl of the supernatant was mixed with 100 µl of the ATP working dilution. ATP amounts were normalized to the protein concentration of the corresponding samples. Protein concentrations were determined by the Bradford analysis (Takara, China).

2.5 Metabolite Extraction from Placental Mitochondria

Metabolites were extracted from the isolated placental mitochondria using cold methanol/water (1:1 v/v) and sonication was repeated 5 times for 10 sec. The internal standard 2,3,3,3-d4-alanine (0.3 μmol) was introduced into every specimen prior to extraction. After centrifuging at 17,000 g for 15 min at 4°C, the supernatant was collected and dried in the SpeedVac (Labconco Corp., Missouri, USA) at room temperature for 5 hours, and kept at −80°C prior to chemical derivatization.

2.6 Chemical Derivatization and Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The SpeedVac-dried specimens were chemically derivatized using the methyl chloroformate (MCF) approach, based on the method published by Smart et al[24]. The MCF derivatives were analyzed in an Agilent GC7890B system coupled to a MSD5977A mass selective detector (EI) set at 70 eV. The GC column installed for metabolite analysis was the ZB-1701 GC capillary column (30 m x 250 μm id x 0.15 μm with 5 m guard column, Phenomenex). The GC analysis parameters were as described by Smart et al[24].

2.7 GC-MS Data Extraction, Data Normalization, and Statistical Analysis

AMDIS software was used to deconvolute GC-MS chromatograms and compounds were identified using the MCF mass spectra library developed by Smart et al[24]. The metabolites were identified according to the MS spectrum of the MCF-derivatized metabolite and the corresponding chromatographic retention time. The relative intensity of identified metabolites was calculated by the XCMS-based R-script with the GC base-peak value of a chosen reference ion within an appropriate retention time bin. The metabolite abundance was normalized by the amount of the internal standard (2,3,3,3-d4-alanine) detected in each sample, and the batch variation was then removed by median centering. Student's t test and false discovery rates (FDR) were performed in the R program to determine whether the relative concentration of each identified metabolite was significantly different between the placentas of the PE and the normal pregnancies. PLSDA and receiver operating characteristic (ROC) curves were conducted using an Excel add-in package called Multibase (Numerical Dynamics, Japan). Our PAPi algorithm was used to estimate and compare the various metabolic pathways in placental mitochondria[25]. Graphical illustrations of the results were constructed using gplots and ggplot2 R packages[26]. Data are expressed as the mean ± standard deviation (SD). Student's t test was applied to compare results from the western blot analysis, using Graph-Pad Prism software (GraphPad Software,La Jolla, California, USA). Student's t test, Mann-Whitney test, and chi-squared test were used to compare the clinical information between normal and PE groups, using the R program. $p<0.05$ was considered statistically significant.

3. Results

3.1. ATP Levels in the Placentas from the sPE and Normal Groups

The results from the luminescence assay demonstrated that ATP levels in the placentas from the sPE group were significantly decreased by 32.4% compared to the standard group (p-value < 0.01; Figure 1).

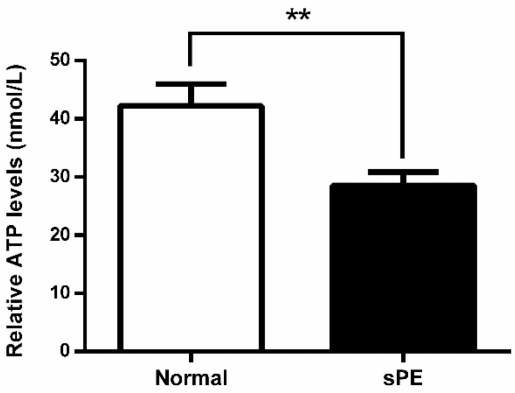

Figure 1. Total ATP levels in placental villous tissues. ATP levels decreased significantly in sPE placentas compared with normal placentas. sPE: severe preeclampsia.

3.2. Metabolite Profiles of the Isolated Placental Mitochondria in sPE and Normal Pregnancies

We detected a total of 139 GC-MS peaks in the placental mitochondria, 92 of which were accurately identified using the Villas-Bôas MCF MS library (**Table S1**). For multivariant analysis of all GC-MS features, Partial least square discriminant analysis (PLSDA) showed that the sPE and normal pregnant groups were clustered separately; principal component (PC)1 and PC2 explained 61.6% and 7.8% of the variance, respectively (**Figure 2A)** and the validation parameters of six PCs were as follows: R2 = 0.96 and Q2 = 0.77 (**Figure 2B**). The univariate analysis revealed 40 metabolites that were significantly different between the sPE and the normal placental mitochondria (p-values and q-values < 0.05; **Figure3**). The majority of the metabolites were detected at higher concentrations in samples from placental mitochondria of sPE pregnancies. The metabolites were comprised of a range of intermediates from the central carbon metabolism involving 11 amino acids, 13 unsaturated fatty acids, 10 saturated fatty acids, 1 glycolytic intermediate, 2 amino acid derivatives, and 3 other metabolites. In contrast, citraconate and caprylate were detected at significantly lower concentrations in sPE placental mitochondria (**Figure 3**). Interestingly, there were 5 fatty acids with an area under the Receiver operating characteristic (ROC) curve above 90%; these included omega-6 polyunsaturated fatty acids (e.g. arachidonate, bihomo-γ-linoleate, and γ-linoleate), an omega-3 polyunsaturated fatty acid (e.g. docosapentaenoate (DPA)), and an unsaturated fatty acid (e.g. myristate) as illustrated in **Figure 4**.

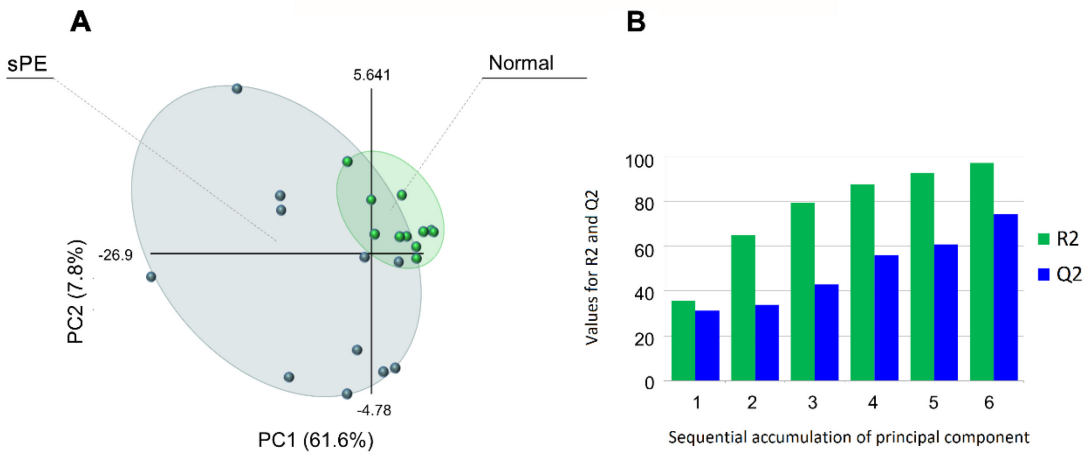

Figure 2. Partial least square discriminant analysis (PLSDA). A) Principal component 1 (PC1) vs. PC2 score plot reveals that placental mitochondria from sPE (blue) and normal (green) pregnancies are clustered separately. B) Leave-one-out cross-validation of established PLSDA model with R2 = 0.96 and Q2 = 0.77 for accumulated six principal components.

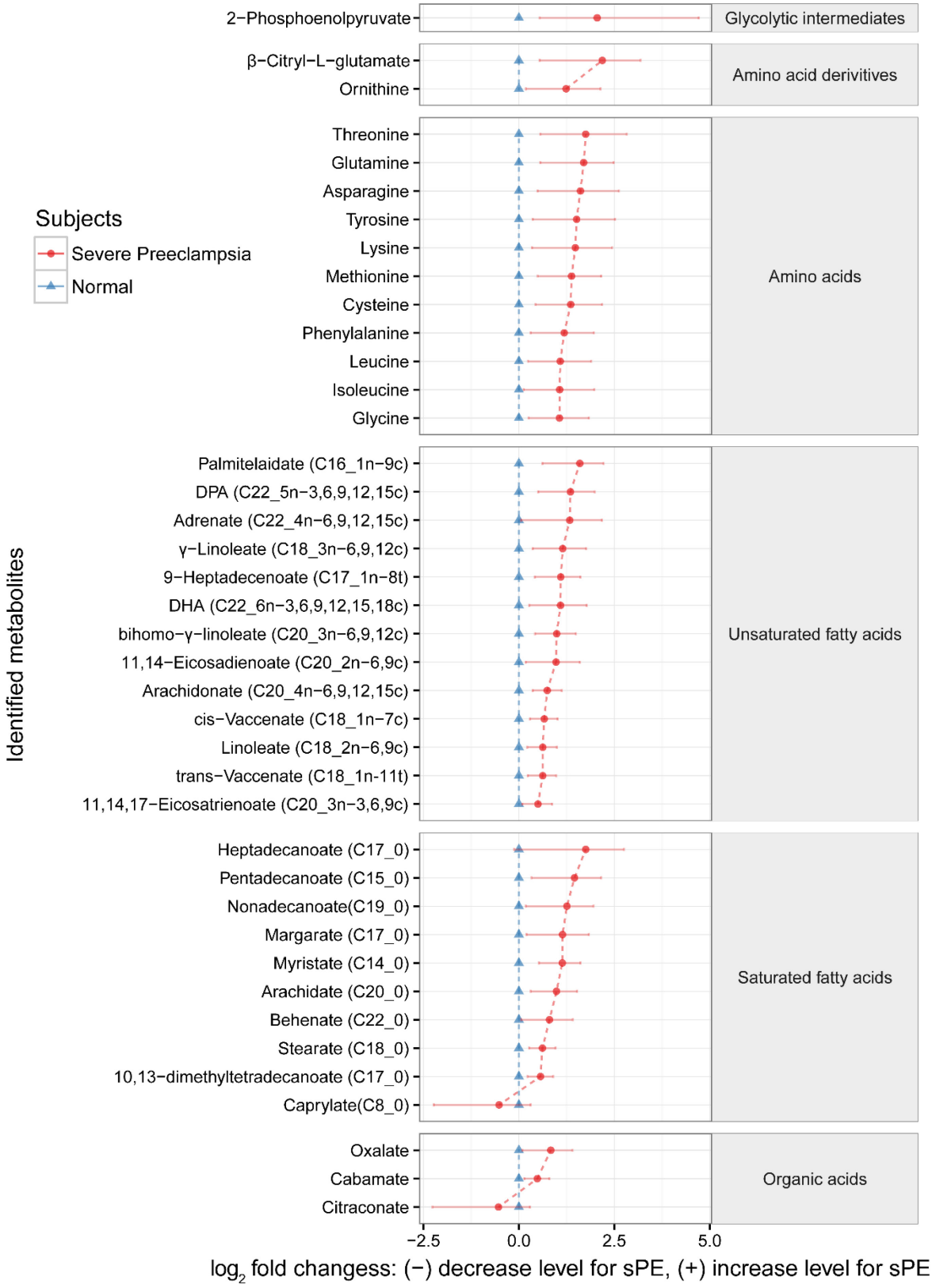

Figure 3. The ratio of identified metabolite concentrations in placental mitochondria in sPE and normal

pregnancies. Blue triangles (▲) represent the mean metabolite concentrations in placental mitochondria from normal pregnant women that were adjusted to 0. Red circles (●) indicate metabolite concentrations in placental mitochondria from women diagnosed with sPE. The relative abundance of metabolites was plotted using $\log_2$ scale. The 95% confidence interval (⟷) for a quotient of two means (sPE/Control) is calculated by Fieller's approach. Only metabolites with statistically significant differences in concentration and low false discovery rate ($p < 0.05$ by Student's t test, $q < 0.05$ by false discovery rate) are illustrated.

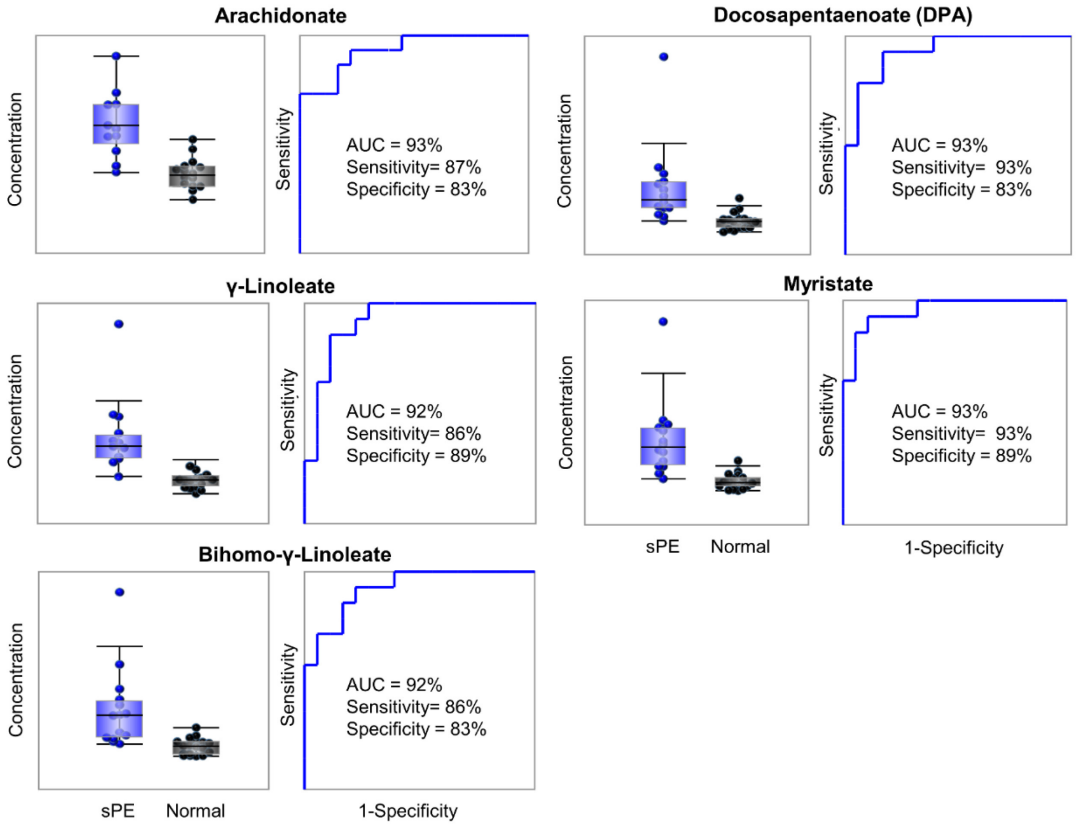

Figure 4. ROC curves for five fatty acids with an area under the ROC curve above 90 %; comparisons are between sPE and normal pregnancies.

3.3. Prediction of Metabolic State of Placental Mitochondria in sPE and Normal Pregnancies

By comparing the placental mitochondrial metabolome in sPE and normal pregnant placentas, we generated a comparative metabolic activity profile via the Pathway Activity Profiling (PAPi) R package. Figure 5 demonstrates the metabolic activities of placental mitochondria in the normal and sPE subjects. In contrast to the healthy pregnancies, 21 metabolic pathways appeared to have been downregulated in placental mitochondria from the sPE pregnancies with p-values and q-values < 0.05. These represent a range of pathways including the metabolism of lipids, amino acids, cofactors, endocrine metabolites, vitamins, secondary metabolites, and metabolites involved in the immune system. In particular, four of the identified metabolic pathways were related to lipid metabolism with p-values and q-values <0.01. Two of the pathways are involved in omega-6 polyunsaturated fatty acid metabolism, which leads to the production of prostaglandins; linoleic acid metabolism and arachidonic acid metabolism.

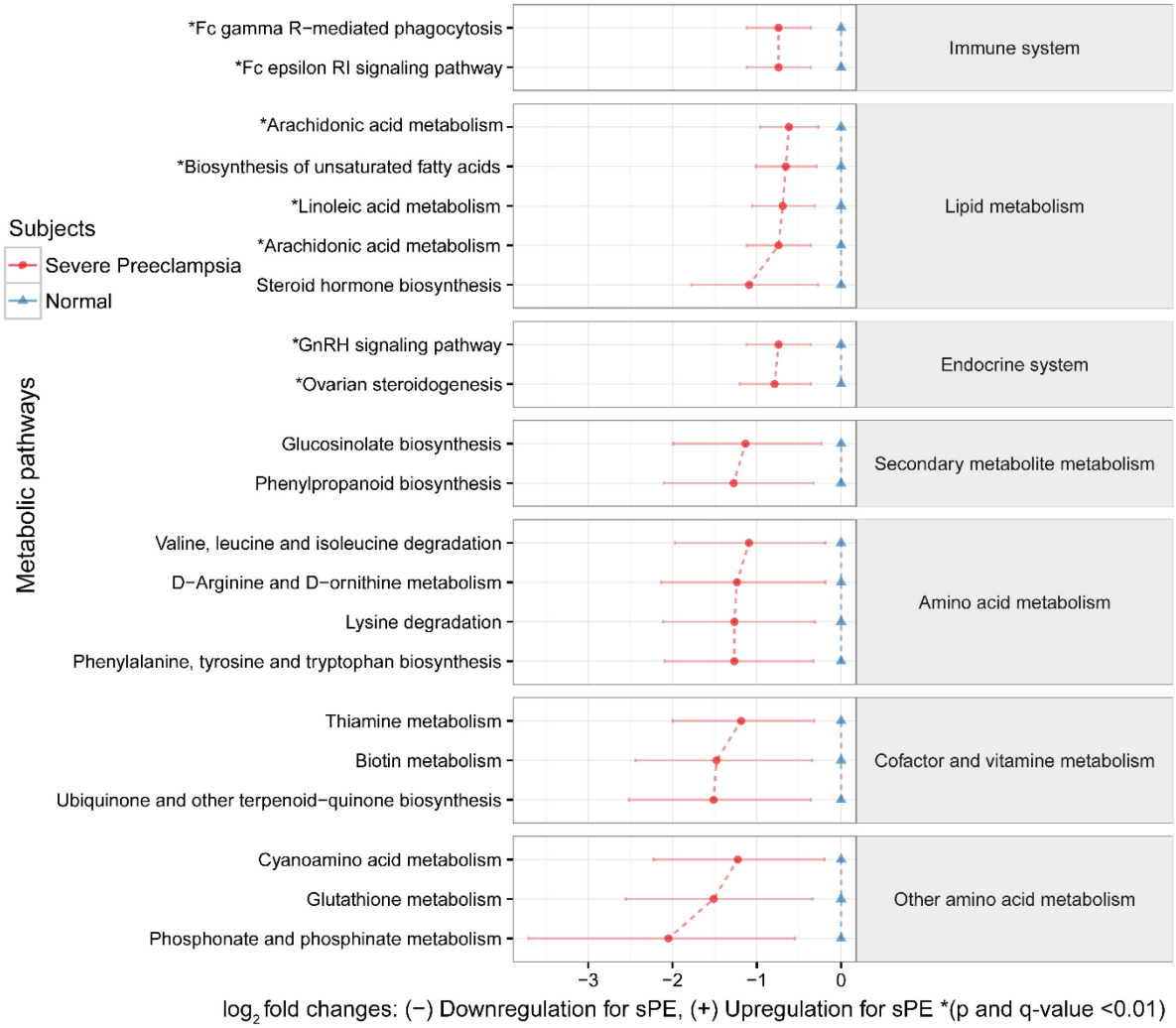

Figure 5. Activities of metabolic pathways based on the placental mitochondrial metabolome of sPE and normal pregnancies. Blue triangles (▲) represent metabolic activities in placental mitochondria from normal pregnant women that were adjusted to 0. Red circles (●) represent metabolic activities in placental mitochondria from women diagnosed with sPE. The metabolic activities were visualized using $\log_2$ scale. Negative values mean the metabolic pathway activity was downregulated. The 95% confidence interval (⟷) for a quotient of two means (sPE/Control) is calculated by Fieller's approach. Only the pathways showing statistically significant Student's t test ($p < 0.01$) and minimum false discovery rate ($q < 0.01$) are shown.

3.4. Association of Abnormal Metabolism with Distorted Mitochondrial Dynamics, Disordered Degradation, and Diminished Biogenesis

We used Western blots to investigate the expression of proteins included in the modulation of mitochondrial dynamics, biogenesis, and mitophagy. By comparison with the normal placentas, the expression of the mitochondrial fusion-related proteins Mitofusin 1 (MFN1), MFN2, and Optic Atrophy 1 (OPA1) were found to be downregulated in the sPE placentas, while no statistical differences were observed in the fission-related proteins Dynamin 1-like (DRP1) and Fission 1 (Fis1) (Figure 6A and B). BNIP3-induced mitophagy, a macroautophagy involved in maintaining cellular well-being by discerning surrounding compromised and depolarized mitochondria under hypoxia, was shown to be inhibited in sPE (Figure 6C). Furthermore, the reduced expression of PGC-1α and SIRT3, proteins involved in mitochondrial biogenesis was also observed in PE (Figure 6D).

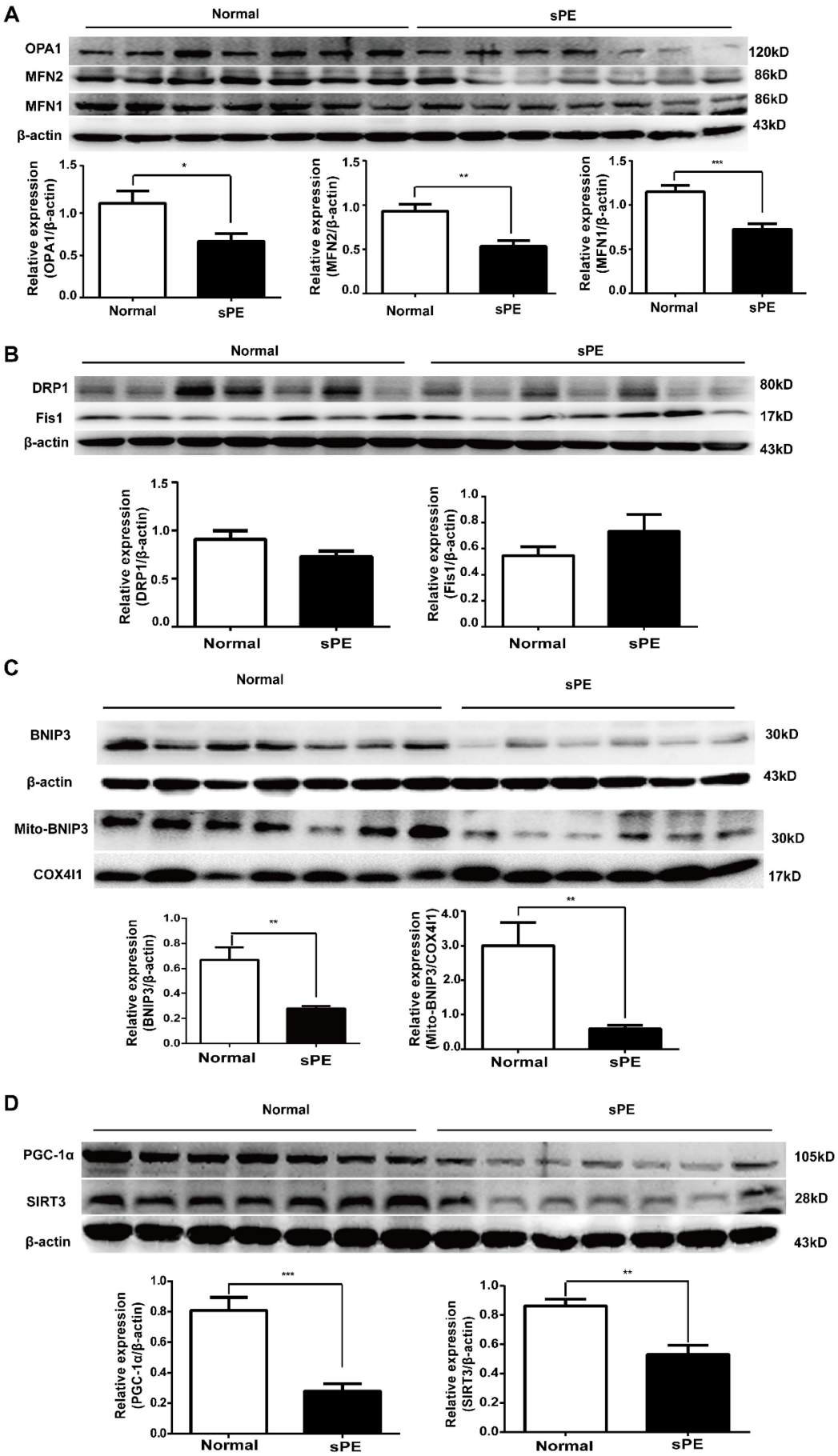
A
Normal
sPE
OPA1
MFN2
MFN1
β-actin
120kD
86kD
86kD
43kD
Relative expression (OPA1/β-actin)
Relative expression (MFN2/β-actin)
Relative expression (MFN1/β-actin)
Normal
sPE
B
DRP1
Fis1
β-actin
80kD
17kD
43kD
Relative expression (DRP1/β-actin)
Relative expression (Fis1/β-actin)
C
BNIP3
β-actin
Mito-BNIP3
COX4I1
30kD
43kD
30kD
17kD
Relative expression (BNIP3/β-actin)
Relative expression (Mito-BNIP3/COX4I1)
D
PGC-1α
SIRT3
β-actin
105kD
28kD
43kD
Relative expression (PGC-1α/β-actin)
Relative expression (SIRT3/β-actin)

Figure6. Protein expression profiles of proteins involved in mitochondrial fusion, fission, degradation, and biogenesis in placentas. A) Western blotting confirmed the decreased expression of mitochondrial fusion-associated proteins, including OPA1, MFN2, and MFN1 in sPE placental tissue. β-actin was used as a loading control. B) No significant difference was found in mitochondrial fission in sPE placental tissue. C) The expression of BNIP3, extracted from placentas, was decreased in sPE. β-actin was used as a loading control. The expression of Mitochondrial BNIP3 (Mito-BNIP3), extracted from placental mitochondria, was reduced in sPE. COX4I1 was used as a loading control. D) The expressions of mitochondrial biogenesis associated mediators, including PGC-1α and Sirt3, were substantially lowered in sPE. β-actin was used as a loading control. * = p <0.05, ** = p < 0.01, *** = p < 0.001.

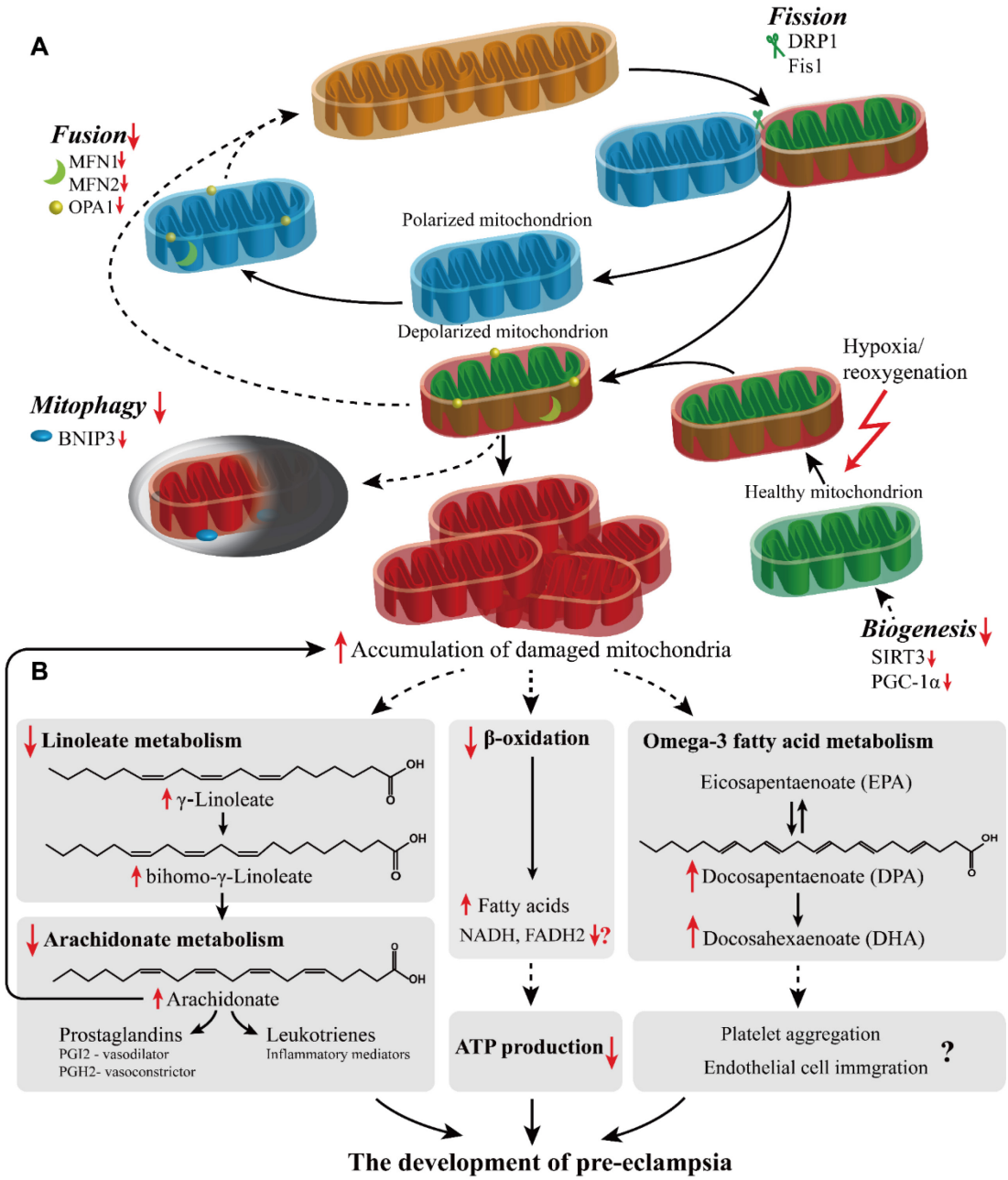

Figure 7. Proposal as to how mitochondrial disorders and metabolic pathways discussed in this study might be involved in the pathophysiology of PE. A) Distorted mitochondrial fusion, disordered mitophagy, and diminished mitochondrial biogenesis are three major mitochondrial malfunctions in PE. Upon the damage of mitochondria, the fusion event maintains genetic and biochemical homogeneity by

enabling the dilution of toxic superoxide species and mutant mtDNA as well as a complementary rescue mechanism. In contrast, fission serves to separate the fused mitochondrion into polarized mitochondria (blue) and depolarized mitochondria (green matrix and red membrane), which, in turn, removes the sustained depolarized mitochondria (red) by autophagy – the process is known as mitophagy. The proteins involved in fusion (MFN1, MFN2, and OPA1) and mitophagy (BNIP3) were downregulated in PE, but not in fission-related proteins (DRP1 and Fis1). In addition, PGC-1α and SIRT3, which promote the biogenesis of healthy mitochondria, were also downregulated in PE. All the mitochondrial disorders add to the accumulation of damaged mitochondria. The dashed lines indicate that the direction of the next step is compromised. Upward red arrows represent upregulation, while downward red arrows represent downregulation. B) The accumulation of damaged mitochondria causes imbalanced placental metabolism that affects linoleate, arachidonate, and omega-3 fatty acid metabolism, and β-oxidation. These metabolic disturbances may lead to reduced ATP production, inflammation, vasoconstriction/vasodilation, impaired endothelial cell immigration, and attenuated platelet aggregation, which are all features of PE. The accumulation of arachidonate also induces further mitochondrial damage. Finally, only the chemical structure of identified metabolites with statistically significant differences between PE and normal pregnancies are shown.

4. Discussion

This study was the first to apply metabolomic and Western blot approaches to elucidate how mitochondrial dysregulation can change placental metabolism during the development of sPE. We observed a reduced level of ATP in sPE, a finding that was consistent with previous studies[27, 28]. We also detected a higher level of fatty acids (**Figure 3**) and reduced fatty acid catabolism **(Figure5)** in placental mitochondria from sPE pregnancies. Since β-oxidation is the catabolism of fatty acid into NADH, FADH2, and acetyl-CoA, which then feeds into the TCA cycle, the potential attenuation of β-oxidation may have contributed to the accumulation of fatty acids and reduced ATP production observed in this study. In accord with previous studies, the expression of metabolic enzymes involved in the initial step of the β-oxidation pathway, including the hydroxyacyl-CoA dehydrogenase α subunit, the hydroxyacyl-CoA dehydrogenase β subunit, and long chain acyl-CoA dehydrogenase, was found to be reduced in PE[29-31]. Other researchers have also reported that dyslipidemia contributes to the development of PE[32, 33]. Together, these findings suggest that the lower levels of the ATP pool in placentas from PE pregnancies may be the result of β-oxidation downregulation.

Higher concentrations of intermediates from omega-6 fatty acid metabolism - γ-linoleate, bihomo-γ-lineolate, and arachidonate - were detected in the placental mitochondria of women that developed PE in our study (**Figure 3**). Interestingly, γ-linoleate is converted into bihomo-γ-linoleate by elongase and then desaturated to arachidonate by Δ-5 desaturase, which, in turn, is the precursor for the biosynthesis of prostaglandins and leukotrienes (**Figure 7B**). Prostaglandins can be vasodilators (e.g. PGI2p) and vasoconstrictors (e.g. PGH2p), and leukotriene is an inflammatory mediator that is synthesized in leukocytes or other immune cells by the oxidation of arachidonate. Leukotriene increases vascular permeability and promotes the adhesion of leukocytes to the endothelium. Both vasoconstriction and an inflammatory response are known to contribute to the pathogenesis of PE. In addition, higher levels of arachidonate seem to have adverse effects on mitochondria. Cocco *et al*. showed that arachidonate interacts with the mitochondrial electron transport chain and induces reactive oxygen species production[34]. Scorrano *et al*. demonstrated that arachidonate promotes apoptosis via the mitochondrial permeability transition pore[35]. In addition, Penzo *et al*. proposed that arachidonate

released by phospholipase A_2 cleavage induces Ca^{2+}-dependent apoptosis via the mitochondrial caspase cascade[36]. Thus, the accumulation of arachidonate inside mitochondria may lead to further mitochondrial damage.

Docosapentaenoate (DPA) is another polyunsaturated fatty acid likely to be involved in the development of PE. There are two isomers of DPA (abbreviated as n-3 DPA and n-6 DPA). n-3 DPA is an omega-3 fatty acid and can act as an intermediate metabolite between eicosapentaenoate (EPA) and docosahexaenoate (DHA), while n-6 DPA is an omega-6 fatty acid synthesized by the stepwise elongation and desaturation of arachidonate. Our GC-MS analysis only identified higher concentrations of n-3 DPA in PE; *in vivo* studies have demonstrated that n-3 DPA can be retro-converted back to EPA, but that there is limited catabolism of n-3 DPA to DHA[37, 38]. n-3 DPA has been shown to promote endothelial cell migration in response to fetal bovine serum[39] and inhibited angiogenesis in aortic endothelial cells via suppression of vascular endothelial growth factor (VEGF)[40]. In addition, n-3 DPA has been shown to reduce platelet aggregation[41]. These results lead us to propose a potential connection between n-3 DPA and the failure of maternal endothelial remodeling and hypertension that occurs in the pathophysiology of PE.

A paucity of information regarding mitochondrial dysfunction in PE, including the nature of the contribution of omega fatty acid metabolism to the disease, led us to investigate candidate proteins which may influence mitochondrial metabolism, using Western blots. Interestingly, our results indicated that MFN1, MFN2, OPA1, BNIP3, PGC-1α, and SIRT3 were all downregulated in sPE (**Figure 6**). Based on these findings, we propose that there are three major mitochondrial malfunctions in sPE: 1) distorted mitochondrial fusion; 2) disordered mitophagy; and 3) diminished mitochondrial biogenesis (**Figure 7A**).

Mitochondrial fusion is crucial to mitochondrial well-being; fusion enables functional complementation between mitochondria through exchanges of proteins and mtDNA nucleoids[42]. Deficient mitochondrial fusion is particularly deleterious, as it results in reduced energy production, excessive oxidative stress, increased mutation rates, and mtDNA deletion[43-45]. Our results identified that proteins associated with fusion including MFN1, MFN2, and OPA1 were downregulated in sPE. No differences were observed in the fission-related proteins such as Fis1 and DRP1. These findings are inconsistent with the findings of Vishnyakova *et al* who reported that OPA1 was upregulated in PE placentas[46], while Yu *et al* also found that downregulation of MFN2 was related to PE[27]. The findings of Vishnyakova *et al* were mainly based on gene expression. Levels of DNA or RNA may be unable to predict protein levels accurately, as they do not account for post-transcriptional/translational modifications[47-49]. In addition, Yu *et al* demonstrated that ATP was significantly reduced after knockout of the MFN2 gene in trophoblast cells[27]. In summary, our findings implicate fusion but not fission in the abnormal mitochondrial function of PE.

The downregulation of BNIP3 observed in our study implicates attenuated mitophagy in PE placentas. Several studies have reported the importance of the BNIP3 gene family in mitochondrial quality control through the mediation of mitophagy. For instance, a study exploring the effects of mitophagy on mammary tumors, discovered that BNIP3 impedes main mammary tumor development and advancement by halting the accumulation of impaired mitochondria and the consequent excess ROS generation[50]. Furthermore, both β-oxidation of fatty acid and oxidative phosphorylation were attenuated in associated with a BNIP3 gene defect[51, 52]. Interestingly, our data demonstrated that mitophagy induced by BNIP3 was suppressed in PE, despite our previous publication having identified excessive autophagy in

PE placentas[53]. Consistent trends have been observed in research into the pathogenesis of strokes; a deficiency in BNIP3 substantially lowered neuronal mitophagy but strengthened nonselective autophagy after ischemic/hypoxic insults[54]. Thus, the abnormal mitophagy induced by BNIP3 may be related to the accumulation of damaged mitochondria, excessive production of ROS, and compromised lipid/energy metabolism in PE.

PGC-1α and SIRT3 downregulation may contribute to diminished mitochondrial biogenesis and compromised lipid metabolism. PGC-1α was found to be the master regulator of mitochondrial biogenesis in brown adipose tissue and skeletal muscle[55]. Abnormal mitochondrial biogenesis from SIRT3 dysfunction results in poor developmental outcomes for human oocytes after in vitro maturation[14]. Knock-out of SIRT3 lead to compromised β-oxidation and accumulation of β-oxidation intermediates in liver and skeletal muscle[56, 57]. In this study, we identified a significant reduction of PGC-1α and SIRT3 expression in sPE placentas and altered lipid metabolism in mitochondria. Our results are similar to Poidatz's findings that mRNA expression of inducers of mitochondrial biogenesis, estrogen-related receptor-γ, SIRT1 and PGC-1α, was downregulated in PE placentas[58]. These findings suggest PGC-1α and SIRT3 play a role in the pathophysiology of PE through the dysregulation of mitochondrial biogenesis and fatty acid oxidation.

5. Conclusions

Mitochondrial fusion, autophagy, and biogenesis appear to be dysregulated in the placenta of women that developed PE. Moreover, metabolic dysfunction of the mitochondria from sPE is reflected in the placental mitochondrial metabolome. The altered lipid metabolism indicates that mitochondrial fusion, autophagy, and biogenesis are potentially to modulating the intrinsic activity of catabolic enzymes to oxidize substrates to synthesize ATP. The higher concentrations of polyunsaturated omega-3 and omega-6 fatty acids in mitochondria, such as DPA, arachidonate, and linoleate may be involved in the dysregulation of PE placentas. Future research is needed to validate the findings of our study.

Conflicts of Interest: There are no conflicts of interest.

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