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Research paper

Targeting trophoblast cell mitochondrial dysfunction in preeclampsia via drug repurposing

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ABSTRACT

Preeclampsia is a multifactorial pregnancy disorder characterized by the new onset of hypertension and organ damage. Mitochondrial dysfunction is central to preeclampsia pathogenesis leading to placental dysfunction and oxidative stress. This study aims to elucidate the mechanisms of mitochondrial dysfunction in first-trimester trophoblast cells and to assess the therapeutic potential of aspirin, metformin, resveratrol, and a FKBPL-based peptide (AD-01) as a strategy to improve trophoblast mitochondrial health. A 2D *in vitro* model using the first trimester ACH-3Ps trophoblasts were developed to mimic preeclampsia-like conditions, including hypoxia-inducible factor (HIF)-1α activation (DMOG, 100 μM), mitochondrial dysfunction (Rho-6G, 1 μg/mL), or inflammation (TNF-α, 10 ng/ml). Cells were treated for 48 h with metformin (0.5 mM), resveratrol (15 μM), AD-01 (100 nM), or aspirin (0.5 mM), in the presence of DMOG, Rho-6G or TNF–α. Mitochondrial dynamics were assessed by immunofluorescence staining, the Seahorse XF Mito Stress Test, and RT-qPCR for key genes expression regulating mitochondrial fusion (*mfn1*), fission (*dnm1l*), and autophagy (*atg5, map1lc3b*). Preeclampsia-mimicking stimuli significantly altered mitochondrial networks by reducing mitochondrial size ($p < 0.05$-0.0001), increasing circularity ($p < 0.05$-0.0001), and decreasing mitochondrial number per cell ($p < 0.0001$). Metformin notably restored mitochondrial architecture under inflammatory stress, normalized *mfn1* ($p < 0.05$) and *atg5* expression ($p < 0.001$), and improved cellular bioenergetics. Aspirin improved mitochondrial morphology under hypoxic conditions and reduced oxygen consumption ($p < 0.01$). Resveratrol and AD-01 showed context-dependent protective effects, including reduced basal respiration under inflammatory stress ($p < 0.0001$). These findings demonstrate that hypoxia, inflammation, and mitochondrial dysfunction contribute to mitochondrial pathology in preeclampsia and highlight aspirin, metformin, resveratrol, and AD-01 as promising targeted therapies. Tailored interventions may improve mitochondrial health and pregnancy outcomes in women with preeclampsia.

1. Introduction

Preeclampsia is a heterogeneous, multisystem disorder that arises in the second half of pregnancy. It is defined by the new-onset of hypertension (blood pressure >140/90) and proteinuria after 20 weeks of gestation, and it can affect multiple organ systems, including the renal, hepatic, neurological, and cardiovascular systems [1,2]. Preeclampsia is a leading cause of maternal and perinatal morbidity and mortality in pregnancy worldwide; however, it can also increase the risk of future cardiovascular and metabolic diseases [1,3–5]. Monitoring options for preeclampsia remain limited, and the only definitive treatment is delivery of the baby and placenta. When delivery must occur preterm, it can lead to significant maternal and neonatal complications [2,6]. Although the aetiology of preeclampsia is not yet fully understood,

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placental dysfunction is considered the root cause, with mitochondrial dysfunction being the main contributor [7–11].

Mitochondria play a crucial role in placental development and growth, particularly within the syncytiotrophoblasts, a specialized cell layer essential for maternal-fetal interactions throughout pregnancy [12,13]. Mitochondrial oxidative phosphorylation (OXPHOS) is crucial for adenosine triphosphate (ATP) production, supporting trophoblast proliferation, invasion, and vascular remodelling during early placental development. Disruptions in OXPHOS can lead to reduced ATP production, compromising placentation [14,15]. Mitochondria are also vital for nutrient transport and metabolism in the placenta [16]. Dysregulation of these metabolic processes can impair syncytiotrophoblast function, hindering nutrient and gas transport and affecting placental growth [16–19]. Beyond energy production, mitochondria regulate oxidative stress and apoptosis in placental trophoblasts; their impaired function can trigger excess reactive oxygen species (ROS) generation and cellular damage leading to inappropriate placentation [8,9,20,21].

The mitochondrial population is regulated not only by the biogenesis of new organelles but also by their selective degradation via mitophagy (e.g., mitochondrial-specific autophagy) [8,22]. Autophagy is essential for maintaining cellular homeostasis by promoting cell survival and eliminating damaged organelles and protein aggregates, hence playing a critical role in supporting normal pregnancy [22].

While there is no definitive treatment for preeclampsia, lifestyle measures, exercise, and adequate rest are often implemented as preventative measures [23,24]. Various pharmacological agents have been investigated for their potential to restore mitochondrial function in preeclampsia [25,26]. Low-dose aspirin (~81–150 mg/day), a non-steroidal anti-inflammatory drug with vasodilatory properties, is an effective prophylactic treatment for preterm preeclampsia (delivery before 37 weeks of gestation) when prescribed before 16 weeks of gestation [27–29]. Metformin, a hypoglycemic agent, and resveratrol, an antioxidant supplement, are currently being repurposed as new treatment options for preeclampsia. AD-01 is a FK506-binding protein-like (FKBPL)-based therapeutic peptide with potent anti-angiogenic activity in cancer through targeting CD44 and DLL4 [30–32]. Recently, AD-01 has demonstrated anti-inflammatory properties by inhibiting the NF-kB pathway and rescuing vascular dysfunction [33]. Interestingly, AD-01 can both positively and negatively regulate FKBPL expression depending on the type of stressor present, restoring its expression to physiological levels [33,34]. FKBPL has a critical role in developmental angiogenesis, and its levels in human plasma and placental tissue are increased in the presence of preeclampsia [35–37]. We have recently shown in similar *in vitro* models that aspirin, metformin, resveratrol and AD-01 can reduce oxidative stress and rescue trophoblast dysfunction under preeclampsia-like stresses [38].

In this study, we used custom-designed 2D *in vitro* first trimester trophoblast cell models of preeclampsia and induced mitochondrial dysfunction by applying specific stressor including inflammation, hypoxia-like or mitochondrial dysfunction. These stressors disrupted mitochondrial networks, dynamics and autophagy, and mitochondrial respiration. We also show, for the first time, that repurposing metformin, resveratrol and AD-01 can effectively target mitochondrial dysfunction in first trimester trophoblasts, providing insights into the mechanisms of action relevant for preeclampsia treatment.

2. Materials and methods

2.1. Cell culture

The ACH-3P first trimester trophoblast cell line, established in 2007, was generously donated by Professor Gernot Desoye (Medical University of Graz, Austria) [39]. ACH-3Ps cells were immortalized by fusing primary trophoblast cells from a 12-week gestation placenta with the choriocarcinoma cell line AC1-1 [39]. ACH-3Ps cells (Ethics: ETH21-6326, University of Technology Sydney) were cultured in Ham's/F12 nutrient mix (Thermo Fisher Scientific, USA) supplemented with 10 % fetal bovine serum (Thermo Fisher Scientific, USA) and 1 % penicillin-streptomycin (P/S) (Thermo Fisher Scientific, USA). ACH-3Ps were used as a first-trimester trophoblast model closely resembling primary trophoblasts [38]. A selection medium containing azaserine (5.7 μM; Sigma-Aldrich, USA) and hypoxanthine (100 μM; Sigma-Aldrich, USA) was applied to cells every two to five passages for 24 h to suppress choriocarcinoma cell growth. Cells were subsequently maintained in standard Ham's/F-12 medium for at least 48 h before downstream experiments. This maintenance protocol minimizes any impact of the selection medium on mitochondrial metabolism. During incubation, cells were maintained at 37 °C in a humidified atmosphere with 5 % CO_2, and ambient oxygen (~21 % O_2), and cultures were routinely tested for mycoplasma contamination. Accutase (Sigma-Aldrich, Germany) was used to dissociate the cells, and experiments were performed at passages P15-25.

2.2. Cell stimuli and treatments

ACH-3Ps were seeded at specific densities depending on the downstream assay type: 3×10^4 cells per fluorodish for immunofluorescence imaging, 4×10^5 cells per well in 6-well plates for RNA extraction and RT-qPCR, and 5×10^4 cells per well in XFe24 flux plates for Seahorse XF Cell Mito Stress Tests. Following cell attachment, cells were incubated overnight in serum-reduced Ham's/F12 medium containing 1 % FBS and 1 % P/S before treatments were added. The serum-reduction step served to partially synchronize cells in the same phase of the cell cycle prior to treatment, thereby minimizing variability in basal metabolic activity and allowing treatment-induced changes in mitochondrial function and gene expression to be more accurately attributed to the experimental conditions. Untreated cells cultured in standard Ham's/F12 medium containing 10 % FBS and 1 % P/S were used as the experimental control (media-only control). This group represents baseline physiological conditions in ACH-3Ps and serves as the negative control in comparison to preeclampsia-like stimuli. The following day, cells were treated for 48 h with one of the following stimuli: dimethyloxalylglycine (DMOG, 1 mM or 100 μM) (Sigma-Aldrich, USA) to activate HIF-1α [40], TNF-α (10 ng/mL) (Sigma-Aldrich, USA) to mimic inflammation [41,42], or Rho-6G (1 μg/ml) (Sigma-Aldrich, USA) to induce mitochondrial dysfunction [43,44]. Initial time-course optimisation experiments comparing 24-, 48-, and 72-h treatment durations indicated that 48 h produced the most relevant changes in oxidative stress markers based on our previous work [38]. DMOG is a cell-permeable prolyl-hydroxylase inhibitor that stabilizes HIF-1α, a master regulator of hypoxia-induced downstream mechanisms. A tri-gas incubator was not employed because the aim was to activate hypoxia-inducible signaling pathways independently of altering ambient oxygen tension. DMOG stabilizes HIF-α under standard incubator conditions (5 % CO_2, atmospheric O_2), allowing controlled and reproducible induction of hypoxia-like molecular responses without the additional variability and logistical constraints associated with low-O_2 culture systems. Rhodamine 6G (Rho-6G) is a cationic dye that acts as a potent inhibitor of mitochondrial OXPHOS. It selectively targets the mitochondrial electron transport chain, impairing ATP synthase-mediated ATP production by blocking proton translocation across the inner mitochondrial membrane. At higher concentrations, it can also cause uncoupled respiration, leading to mitochondrial depolarization and reduced bioenergetic capacity. This compound has been widely used to experimentally induce mitochondrial dysfunction and study bioenergetic deficits in various cell models, including trophoblasts [45]. Each stimulus was applied alone or in combination with metformin [46,47] (0.5 mM) (Sigma-Aldrich, USA), AD-01 [31,48] (100 nM) (MedChemExpress, UK), aspirin [29,43,49] (0.5 mM) (Sigma-Aldrich, USA), resveratrol [50,51] (15 μM) (Sigma-Aldrich, USA). Untreated cells served as controls. Metformin was dissolved in PBS, while aspirin and resveratrol were initially dissolved in DMSO and diluted 10X in PBS to

minimize the DMSO concentration. The final concentration of DMSO in all experiments was ≤0.1 % (v/v), a level generally considered non-toxic to cells. AD-01 was dissolved in sterile water. Drug concentrations were chosen to reflect human plasma levels after absorption, metabolism and distribution [50,52–54].

2.3. Immunofluorescence staining and imaging

ACH-3Ps were seeded onto poly-L-lysine (PLL)-coated fluorodishes (Corning, USA) and exposed to preeclampsia-mimicking stimuli with or without drug treatment as described above. Growth medium was replaced with RPMI containing 10 % FBS and 1 % P/S, before Hoechst33342 (1:4000; Thermo-Fisher) and MitoTracker Deep Red (1:5000; Thermo-Fisher) were added for 30 min to stain nuclei and mitochondria, respectively, prior to confocal imaging.

Live-cell confocal microscopy was performed using a Leica Stellaris 8 with a 63× oil objective (NA 1.4) and 1.518 RI immersion oil. Images (512 x 512 pixels) were acquired at a scanning speed of 600 Hz. Fluorophores were excited at 405 nm (Hoechst 33342) and 641 nm (MitoTracker Deep Red). Emissions were recorded at 431–495 nm using a HyD detector (nuclear Hoechst 33342), and at 647–829 nm using a HyDS detector (mitochondrial MitoTracker Deep Red). To analyse mitochondrial morphology, images were processed in ImageJ/Fiji (National Institutes of Health, USA). The 'Analyze Particles' function was used to measure mitochondrial size (μm2) and circularity (a.u.), excluding particles smaller than 5 pixel2 due to the resolution limit. Total mitochondrial count and count per cell were also calculated. All imaging experiments were performed using three independent biological replicates for each experimental condition.

2.4. Real-time quantitative polymerase chain reaction (RT-qPCR)

RT-qPCR was used to assess mRNA expression of *mfn1, dnm1l, atg5 and map1lc3b* in ACH-3Ps. Total RNA was extracted from cultured cells using TRIsure reagent (Bioline, Australia) according to the manufacturer's instructions. RNA quality and concentration were assessed with a Nanodrop One (Thermo Fisher Scientific). SYBR Green primers were used for amplification (Table 1), and RT-qPCR performed using a Luna One-Step qPCR kit (New England Biolabs, USA) on a CFX96 thermocycler (Bio-Rad) and analysed with CFX Maestro (v 1.0). mRNA expression was calculated using the ΔΔCt method and normalised to ribosomal protein S18. Five independent biological repeats were performed.

2.5. Seahorse XF96 cell Mito Stress Test

ACH-3Ps cells were seeded in XFe24 flux plates (Seahorse) at 0.5 × 10^5 cells/well and allowed to adhere for 6–7 h before the medium was changed to starvation medium (Ham's-F-12 with L-Glutamine, 1 % FBS and 1 % P/S). After overnight incubation, cells were stimulated for 48 h with DMOG (100 μM), Rho-6G (1 μg/mL), TNF-α (10 ng/mL) or media only. Each stimulus was combined with aspirin (0.5 mM), metformin (0.5 mM), AD-01 (100 nM), or resveratrol (15 μM); PBS was used as a control. Prior to conducting the assay, cells were washed twice with PBS (without calcium or magnesium) to remove bicarbonate- and serum-containing culture medium, which can interfere with Seahorse pH and oxygen measurements. These washes were brief to minimize cell stress and did not affect cell viability or mitochondrial function, as confirmed in pilot tests. Plates were equilibrated for 1 h in a non−CO_2 incubator at 37°C. Cellular bioenergetics were analysed using a Seahorse XFe24 extracellular flux analyzer according to the manufacturer's instructions. Oxygen-consumption rate (OCR) was measured in XF medium (non-buffered DMEM with 10 mM glucose, 2 mM L-glutamine and 1 mM sodium pyruvate) under basal conditions and after sequential injections of oligomycin (1 μM; ATP- linked respiration), carbonylcyanide-4-(trifluoromethoxy)-phenylhydrazone (FCCP; 5 μM; maximal respiration) and antimycin A and rotenone (3 μM each; Sigma-Aldrich). Three technical replicates were run for each condition, and a total of 12 measurements of 3 min were acquired. Three independent biological replicates (n = 3) were performed. Further analysis was performed using the Agilent Seahorse XFe Analyzer Wave 2.6 software.

Table 1
Primers used for real-time quantitative polymerase chain reaction.

Primer	Gene Sequence (5′-3′)	Accession Number
rps18 forward	CAGMGGATGTAMGGATGG	NM_022551.2
rps18 reverse	TATTTCTTCTTGGACACACC	NM_022551.2
mfn1 forward	ATCTTTGAGGAGTGTATCTCG	NM_033540.4
mfn1 reverse	GTAGCTAGTATCTGTTTAGCTC	NM_033540.4
dnm1l forward	CTGATTCMTCCGTGATGAG	NM_012062.3
dnm1l reverse	AACCTGTTTAGAGTCCTAGC	NM_012062.3
atg5 forward	AAGACCTTTCATTCAGAAGC	NM_004849.4
atg5 reverse	CATCTTCAGGATCAATAGCAG	NM_004849.4
map1lc3b forward	ATAGMCGATACAAGGGTGAG	NM_022818.4
map1lc3b reverse	CTGTMGCGCCTTCTAATTATC	NM_022818.4

rps18: ribosomal protein s18; mfn1: mitofusin1; dnm1l: dynamin-1-like protein; map1lc3b: microtubule-associated protein 1 *light chain* 3 *beta*

2.6. Statistical analysis

GraphPad Prism (v9.4.0) was used for statistical analysis. Data distribution was assessed using the Shapiro–Wilk normality test prior to statistical comparisons. An ordinary one-way ANOVA with Sidak's multiple comparisons post-hoc test was used to compare groups with normally distributed data. When the data were not normally distributed, a Kruskal-Wallis ANOVA followed by Dunn's multiple comparison post-hoc test was applied. A p-value <0.05 was considered statistically significant. Data were presented as mean ± standard error of the mean (SEM) and as mean ± standard deviation (SD), as indicated.

3. Results

3.1. Preeclamptic stimuli disrupt the mitochondrial network in ACH-3P cell model with limited rescue by aspirin and metformin treatments

We previously showed that exposing ACH-3P first trimester trophoblasts to DMOG, TNF-α or Rho-6G recapitulates oxidative stress in preeclampsia, as measured by intracellular concentration of uric acid (UA) and malondialdehyde (MDA) [38]. In this study, we also showed that all three preeclampsia-mimicking stimuli influence the mitochondrial network architecture in trophoblast cells (Fig. 1). Under physiological conditions (media-only control), mitochondria were abundant, elongated and interconnected, occupying the extensive cytoplasmic space.

As shown in Fig. 1, DMOG and TNF-α caused mitochondria to cluster rather than extend throughout the cytoplasm. Mitochondrial clusters were larger in DMOG-treated cells than in TNF-α-treated cells: they accumulated in the perinuclear region with DMOG treatment, whereas in TNF-α-treated cells the clusters were located toward the cell periphery. Rho-6G treatment disrupted mitochondrial architecture leading to punctate mitochondria. Among treatments, metformin was the most effective at restoring the mitochondrial network in the presence of TNF-α; with confocal imaging showing elongated and interconnected networks more closely resembling control cells. However, metformin did not affect mitochondria in the same manner in the presence of Rho-6G or DMOG (Fig. 1).

3.2. Characteristics of mitochondrial morphology in ACH-3P cells exposed to preeclampsia-like stimuli

In preeclampsia, the disruption of mitochondrial networks within placental cells is a key factor contributing to placental dysfunction [55]. Mitochondria, essential for energy production and regulation of cellular

metabolism, exhibit changes in morphology and function when subjected to stresses associated with preeclampsia or other pregnancy complications, such as hypoxia, mitochondrial dysfunction, and inflammation [56,57]. These disruptions manifest as alterations in mitochondrial size, distribution and shape, which are critical for maintaining cellular homeostasis [26]. Such mitochondrial anomalies are implicated in the pathogenesis of preeclampsia, leading to increased oxidative stress and impaired cellular function within the placenta [25]. Here we investigated how hypoxia, mitochondrial dysfunction or inflammation affect mitochondrial morphology (average size, circularity, and number per cell) in trophoblasts, and whether treatments could mitigate these changes by restoring mitochondrial homeostasis (Supplementary Fig. 1).

In terms of average mitochondrial size, DMOG (100 μM) did not significantly alter mean mitochondrial area relative to control, whereas Rho-6G and TNF-α significantly reduced mitochondria size compared to the control group (Control 6.12 ± 0.30 vs Rho-6G 2.30 ± 0.27vs TNF-α , 4.03 ± 0.81, μm^2, $p < 0.0001$ (Rho-6G), $p < 0.05$ (TNF-α); Fig. 2A). This is consistent with DMOG acting as a hypoxia *mimetic* via HIF stabilization rather than lowering ambient O_2; in our model, DMOG primarily remodeled network features other than mean area. By contrast, DMOG increased mitochondrial circularity (Control 0.54 ± 0.01 vs. DMOG: 0.66 ± 0.02, $p < 0.0001$, Fig. 2B) and reduced mitochondria per cell (Control 25.31 ± 2.00 vs DMOG 15.03 ± 0.68, $p < 0.0001$, Fig. 2C), indicating fragmentation/clustered remodelling without a shift in average size. A higher DMOG dose (1 mM) was cytotoxic and ablated respiration (Supplementary Fig. 2), so 100 μM was used to model sublethal pseudo-hypoxia. Metformin, aspirin or AD-01 treatment did not rescue or further reduce mitochondrial average size (Fig. 2A). However, aspirin restored circularity to control levels (DMOG 0.66 ± 0.02 vs. DMOG + Aspirin 0.57 ± 0.01, $p < 0.01$, Fig. 2B) and partially rescued the number of mitochondrial per cell (DMOG 15.03 ± 0.68 vs DMOG + Aspirin 21.63 ± 2.22, fold change, $p < 0.05$, Fig. 2C).

Mitochondrial circularity also increased significantly under mitochondrial dysfunction (Control 0.54 ± 0.01 vs Rho-6G 0.69 ± 0.02, $p < 0.001$, Fig. 2B) and inflammation conditions (Control 0.54 ± 0.01 vs TNF-α 0.66 ± 0.05, $p < 0.05$, Fig. 2B). These changes indicate a shift from an elongated, rod-like network to a fragmented network composed of short segments or dots. However, metformin, aspirin and AD-01 had no significant effect on circularity in the presence of Rho-6G or inflammatory conditions (Fig. 2B).

Under mitochondrial dysfunction conditions, metformin reduced mitochondria per cell (Rho-6G 24.45 ± 2.73 vs Rho-6G + Metformin 12.78 ± 0.43, $p < 0.05$, Fig. 2C). Under inflammatory conditions, aspirin reduced mitochondria per cell (TNF-α 25.88 ± 1.6 vs TNF-α + Aspirin 16.80 ± 2.40, $p < 0.05$, Fig. 2C).

3.3. Inflammation induces changes in mitochondrial fusion that are restored by metformin

During environmental stress, mitochondria undergo dynamic morphological changes through fusion, fission, and mitophagy to maintain homeostasis, quality control and function [58] Here, we aimed to determine how hypoxia, mitochondrial dysfunction and inflammation affect trophoblast mitochondrial dynamics and mitophagy, and whether metformin, aspirin, AD-01 or resveratrol could reverse these alterations in key mitochondrial gene expression. Expression of the fusion gene, *mfn1,* showed a trend towards downregulation under hypoxia-like conditions (Control 1.00 ± 0.00 vs DMOG 0.54 ± 0.09, fold change, $p = 0.05$, Fig. 3A). Rho-6G treatment also reduced *mfn1* expression (Control 1.00 ± 0.00 vs Rho-6G 0.56 ± 0.08, fold change, $p < 0.01$, Fig. 3A), whereas inflammation increased it (Control 1.00 ± 0.00 vs TNF-α 1.85 ± 0.23, fold change, $p < 0.05$, Fig. 3A). Metformin restored *mfn1* expression under inflammatory conditions (TNF-α 1.85 ± 0.23 vs TNF-α + Metformin 1.04 ± 0.12, fold change, $p < 0.05$, Fig. 3A). In contrast, expression of the fission gene, *dnm1l*, was not altered by any stimulus (hypoxia, mitochondrial dysfunction or inflammation) or by any treatment (metformin, aspirin, AD-01 or resveratrol, Fig. 3B). These RT-qPCR data indicate that hypoxia and Rho-6G downregulate *mfn1* while inflammation upregulates it. Metformin effectively restored *mfn1* under inflammatory conditions, suggesting a potential role in stabilizing mitochondrial dynamics during specific preeclampsia-related stresses.

3.4. Hypoxia and inflammation target mitochondrial autophagy genes that are restored by metformin or resveratrol

Mitochondrial autophagy (mitophagy) is a key quality-control mechanism that eliminates damaged mitochondria, thus maintaining cellular homeostasis. We examined the expression of two critical autophagy-related genes, *atg5* and *map1lc3b,* under preeclampsia-like stresses, with and without treatments in ACH-3P cells. RT-qPCR analysis revealed no significant changes in *atg5* expression under hypoxia-like or mitochondrial dysfunction-induced stresses or after treatments (Fig. 4A). However, inflammation significantly increased *atg5* expression (Control 1.01 ± 0.00 vs TNF-α 1.77 ± 0.27, fold change, $p < 0.01$, Fig. 4A). Both metformin and resveratrol restored *atg5* expression to control levels (TNF-α 1.77 ± 0.27 vs TNF-α + Metformin 0.76 ± 0.05, fold change, $p < 0.001$; and TNF-α + Resveratrol 1.07 ± 0.14, fold change, $p < 0.05$, Fig. 4A). Hypoxia significantly downregulated *map1lc3b* expression (Control 1.00 ± 0.00 vs DMOG 0.35 ± 0.11, fold change, $p < 0.01$, Fig. 4B), whereas metformin restored it (DMOG 0.35 ± 0.11 vs DMOG + Metformin 0.86 ± 0.24, fold change, $p < 0.05$, Fig. 4B). Furthermore, *map1lc3b* expression did not change under mitochondrial dysfunction or inflammation alone; however, metformin reduced *map1lc3b* expression in inflammatory conditions compared with inflammation alone (TNF-α 0.89 ± 0.11 vs TNF-α + Metformin 0.34 ± 0.04, fold change, $p < 0.05$, Fig. 4B). These data highlight metformin's unique ability to modulate autophagy-related gene expression across different stress conditions, underscoring its therapeutic potential for stabilizing mitochondrial dynamics in preeclampsia.

3.5. Cellular bioenergetic tests reveal hypoxia- and inflammation-mediated mechanism on mitochondrial function in ACH-3Ps cells and responses to treatments

Given that mitochondrial dysfunction plays a critical role in preeclampsia pathophysiology [59], we used the Seahorse XF Cell Mito Stress Test to comprehensively assess mitochondrial bioenergetics under preeclampsia-related conditions (hypoxia and inflammation) with or without treatments in ACH-3P cells. This test measures various parameters of mitochondrial function that reflect key aspects of cellular bioenergetics and energy management. A high concentration of DMOG (1 mM) caused significant cell damage, indicating severe impairment of mitochondrial respiration, as shown by ablated oxygen consumption (Supplementary Fig. 2).

In contrast, a lower concentration of DMOG (100 μM) produced respiration similar to PBS-treated cells (Fig. 5A), indicating that cells could adapt to the hypoxia-mimetic condition and meet energy demands. TNF-α -stimulated cells, which mimic inflammatory conditions in preeclampsia, demonstrated higher oxygen consumption and increased maximal respiration (upon FCCP stimulation) compared with media-only controls (Fig. 5A). However, basal oxygen consumption rate (OCR) was similar between TNF-α-treated and media-only cells (Fig. 5B). In DMOG-treated cells, aspirin reduced basal respiration ($p < 0.01$, Fig. 5B) and ATP turnover ($p < 0.05$, Fig. 5C), suggesting less oxygen was required to meet ATP demand; no change in proton leak was observed (Fig. 5D). Aspirin also reduced the maximum respiratory capacity of trophoblast cells exposed to hypoxia ($p < 0.001$) and inflammation ($p < 0.01$) but not under physiological conditions (Fig. 5E), indicating decreased oxygen consumption under stress and potentially reducing cellular overexertion.

We also explored the mitochondrial mechanisms of metformin, AD-

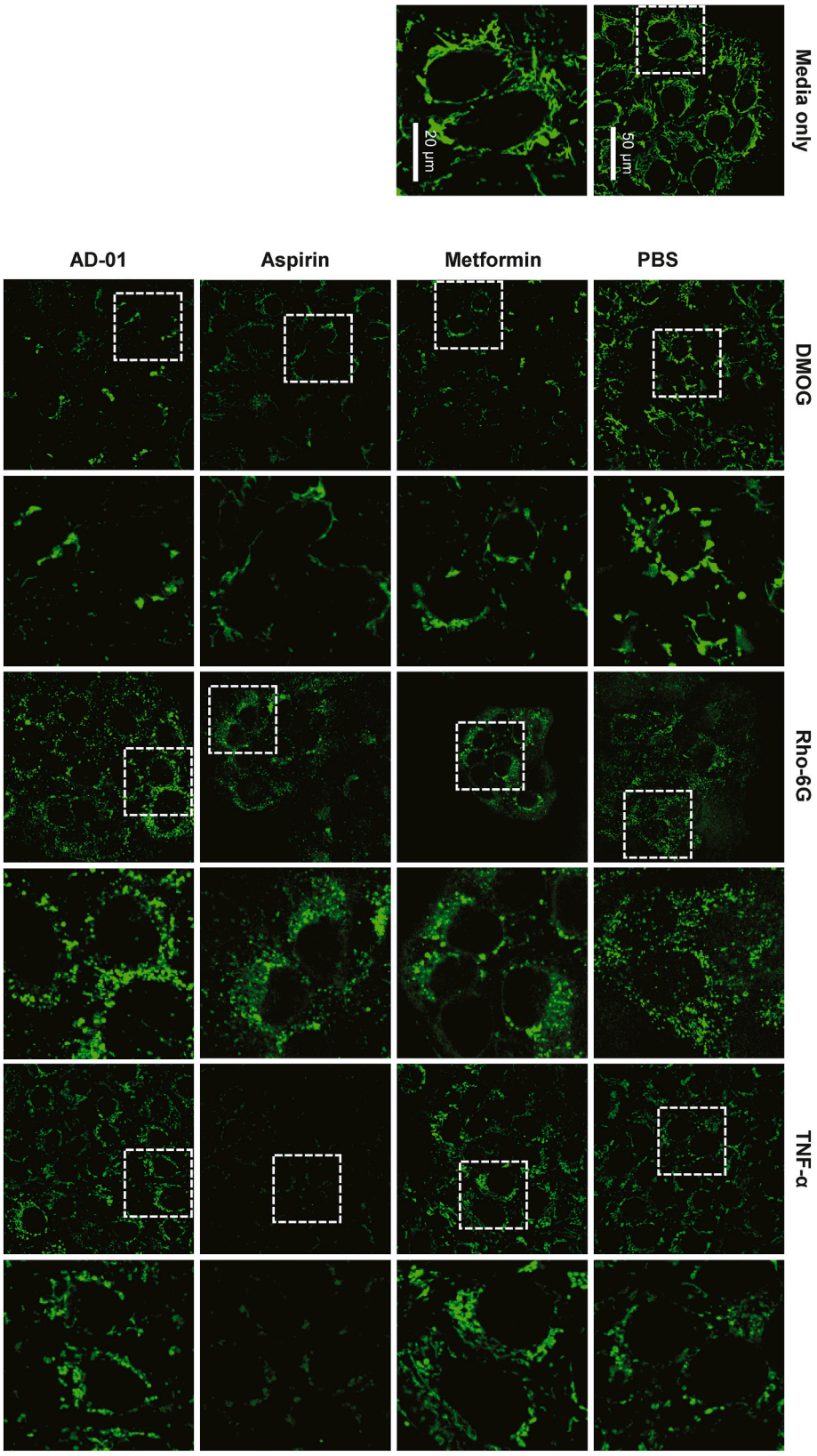

Fig. 1. Impact of HIF-1α, mitochondrial dysfunction and inflammation on mitochondrial networks and morphology in ACH-3Ps cells and their response to treatments. Representative immunofluorescence images show the effects of hypoxia mimetic acting via HIF-1α stabilization (DMOG, 100 µM), mitochondrial dysfunction (Rho-6G, 1 µg/ml) and inflammation (TNF-α, 10 ng/ml) on mitochondrial morphology in ACH-3P trophoblast cells in the presence or absence of metformin (0.5 mM), aspirin (0.5 mM), and AD-01 (100 nM) treatments. (A) Control cells exhibit elongated and interconnected mitochondrial networks; (B) preeclampsia-mimicking stimuli caused mitochondrial fragmentation and clustering. Mitochondria were stained with MitoTracker Deep Red and nuclei were stained with Hoechst 33342. Images were analysed using ImageJ/FIJI "Analyze Particles" function to measure mitochondrial size, circularity, and number per cell. Scale bars represent 50 µm and 20 µm.

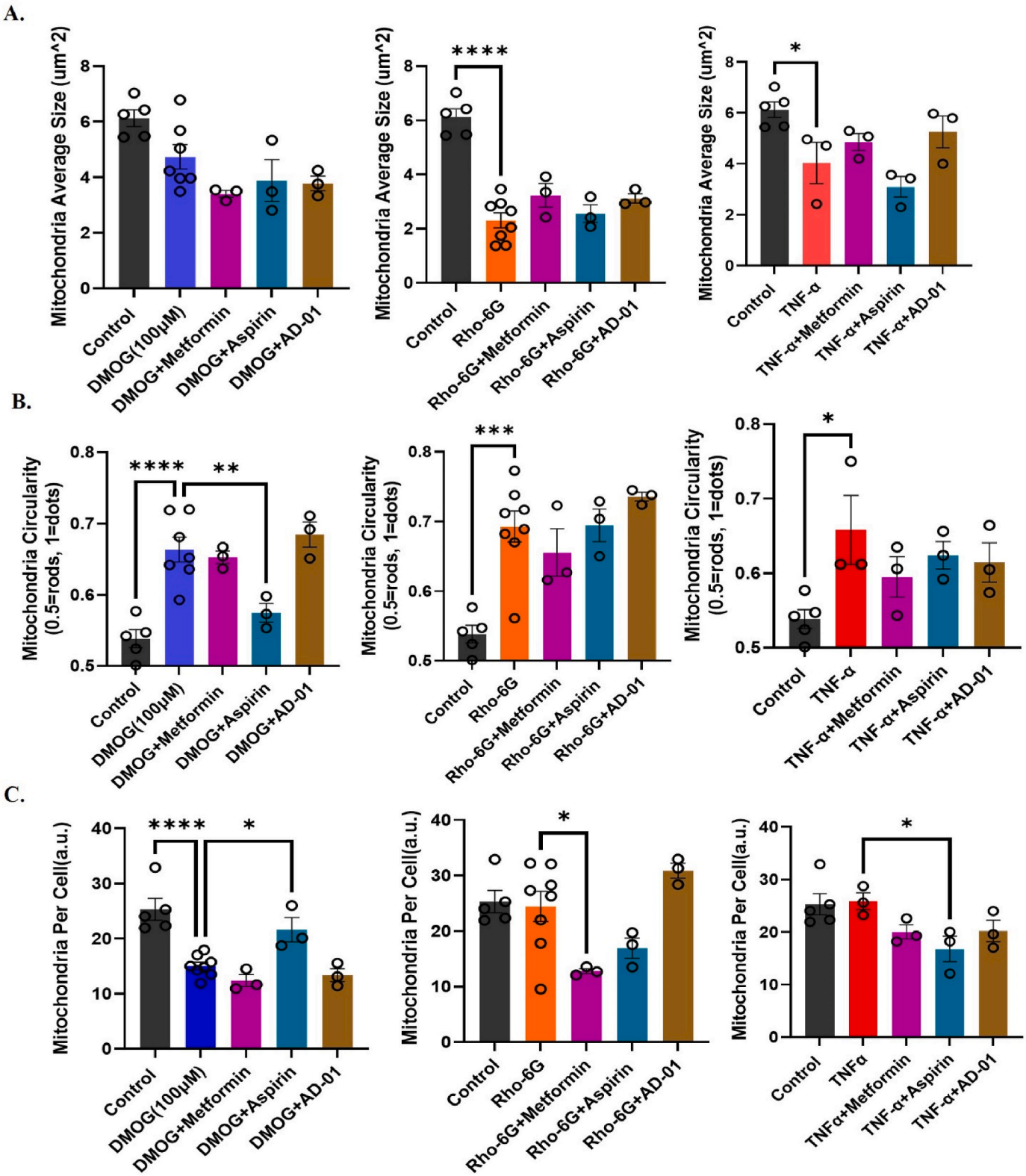

Fig. 2. Preeclampsia-like stimuli impair mitochondrial morphology and networks. ACH-3Ps cells were treated for 48 h with hypoxia (DMOG; 100 μM), mitochondrial dysfunction (Rho-6G; 1 μg/ml), or inflammation (TNF-α; 10 ng/ml) with or without metformin (0.5 mM), aspirin (0.5 mM), or AD-01 (100 nM). Cells in culture media served as untreated controls. Mitochondria were analysed using ImageJ/Fiji 'Analyze Particles' function to quantify: **(A)** average size μm2, **(B)** circularity (=0.5 means rod-like, = 1 means dot-like) and **(C)** number of mitochondria per cell (a.u.). Data are presented as mean ± SEM with individual data points shown where possible. Statistical significance was assessed using one-way ANOVA followed by Sidak's post-hoc test (n = 3–8 biological replicates per group); *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

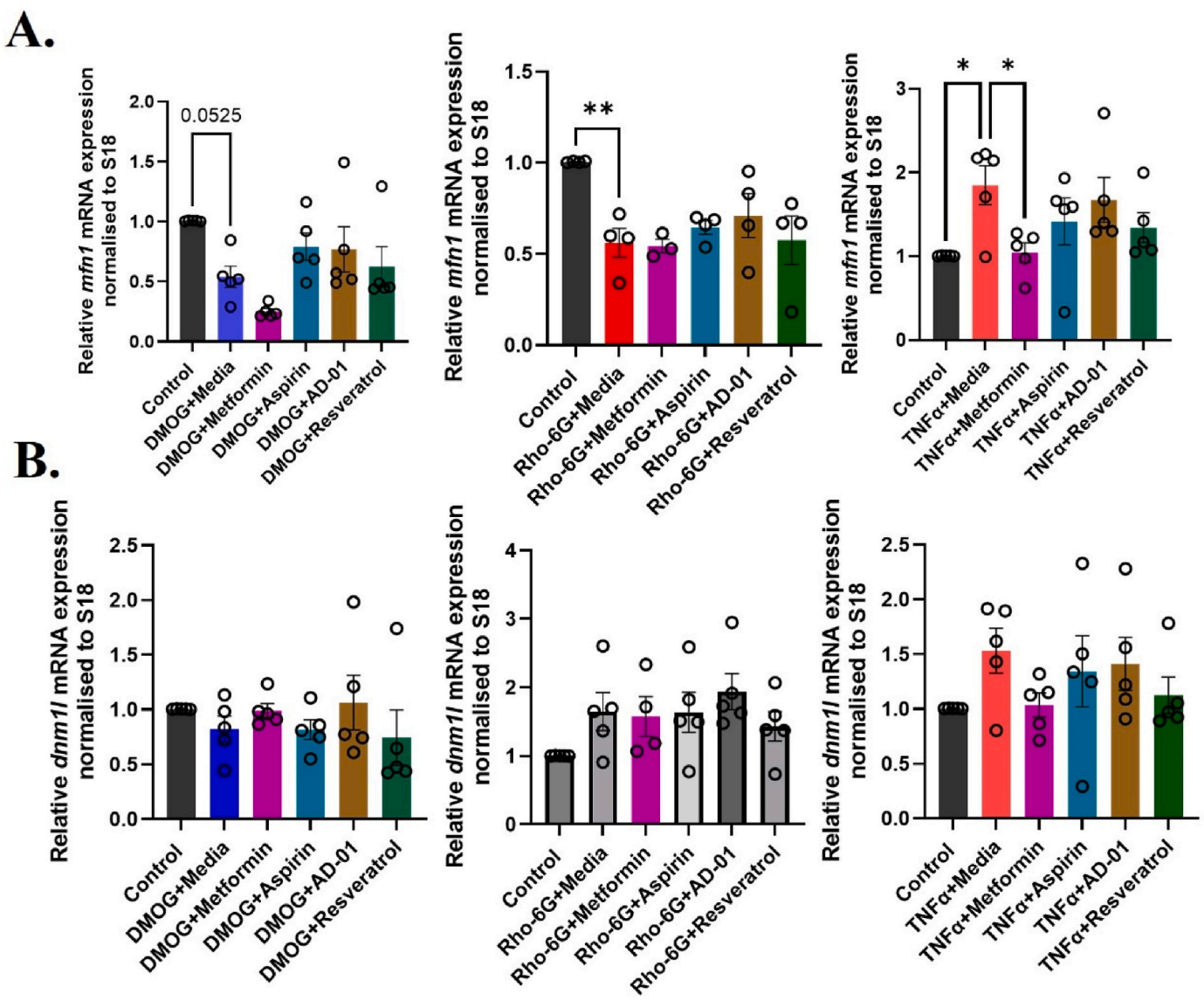

Fig. 3. Preeclampsia-relevant stresses target mitochondrial fusion rather than fission, which is restored by metformin under inflammatory conditions only. ACH-3P cells were treated with (DMOG, 100 μM), (Rho-6G, 1 μg/ml) or (TNF-α, 10 ng/ml) to mimic hypoxia, mitochondrial dysfunction or inflammation, respectively, and were exposed to metformin (0.5 mM), aspirin (0.5 mM), AD-01 (100 nM), or resveratrol (15 μM) for 48 h. Cells in culture media were used as untreated control. Expression of **(A)** the fusion gene mitofusin 1 (*mfn1*) and **(B)** the fission gene dynamin- 1-like (*dnm1l*) was normalised to ribosomal protein S18. Data were normally distributed and analysed by ordinary one-way ANOVA with Sidak's post-hoc test. Data presented as mean ± SEM (n = 3-5 biological replicates); p = 0.05, *p < 0.05, **p<0.01.

01 and resveratrol under hypoxia and inflammation. Under physiological conditions, metformin reduced basal respiration ($p < 0.01$, Fig. 5B), suggesting a lower oxygen requirement for ATP production without a compensatory increase in extracellular acidification rate (ECAR). However, the biological significance of reduced basal respiration in trophoblasts under these conditions remains unclear and requires further functional investigation. Metformin also increased spare respiratory capacity, potentially enhancing the cells' ability to respond to energy demands. In DMOG (100 μM)-stimulated cells, metformin mostly increased respiration (Fig. 5B). Under inflammatory conditions, metformin, AD-01 and resveratrol reduced basal respiration, oxygen consumption for ATP production, protein leak and maximal respiratory capacity ($p < 0.05$–0.0001, Fig. 5B–E). These reductions should be interpreted cautiously, as they may represent either protective metabolic reprogramming or impaired energy production capacity, necessitating further study (Fig. 5A and B). Overall, the Seahorse Mito Stress Test demonstrated that preeclampsia-related stresses, particularly inflammation, cause substantial reductions in mitochondrial respiration, affecting basal respiration and ATP production. These bioenergetic deficits contribute to mitochondrial dysfunction and reflect the cellular energy challenges faced by trophoblasts in preeclampsia.

4. Discussion

In this study, we demonstrate that preeclampsia-related stresses disrupt mitochondrial morphology and dynamics through distinct mechanisms, notably impacting mitochondrial fusion and autophagy. These alterations correlate with bioenergetic strain including higher oxygen consumption and increased maximal respiration, particularly under inflammatory conditions. Consistent with the central role of mitochondrial quality control, ligustrazine nano-drug delivery targeting the PIEZO1–TMBIM6–PHB2 axis improves doxorubicin-induced mitochondrial injury by enhancing PHB2-dependent mitochondrial quality surveillance and suppressing cardiomyocyte pyroptosis [60], while

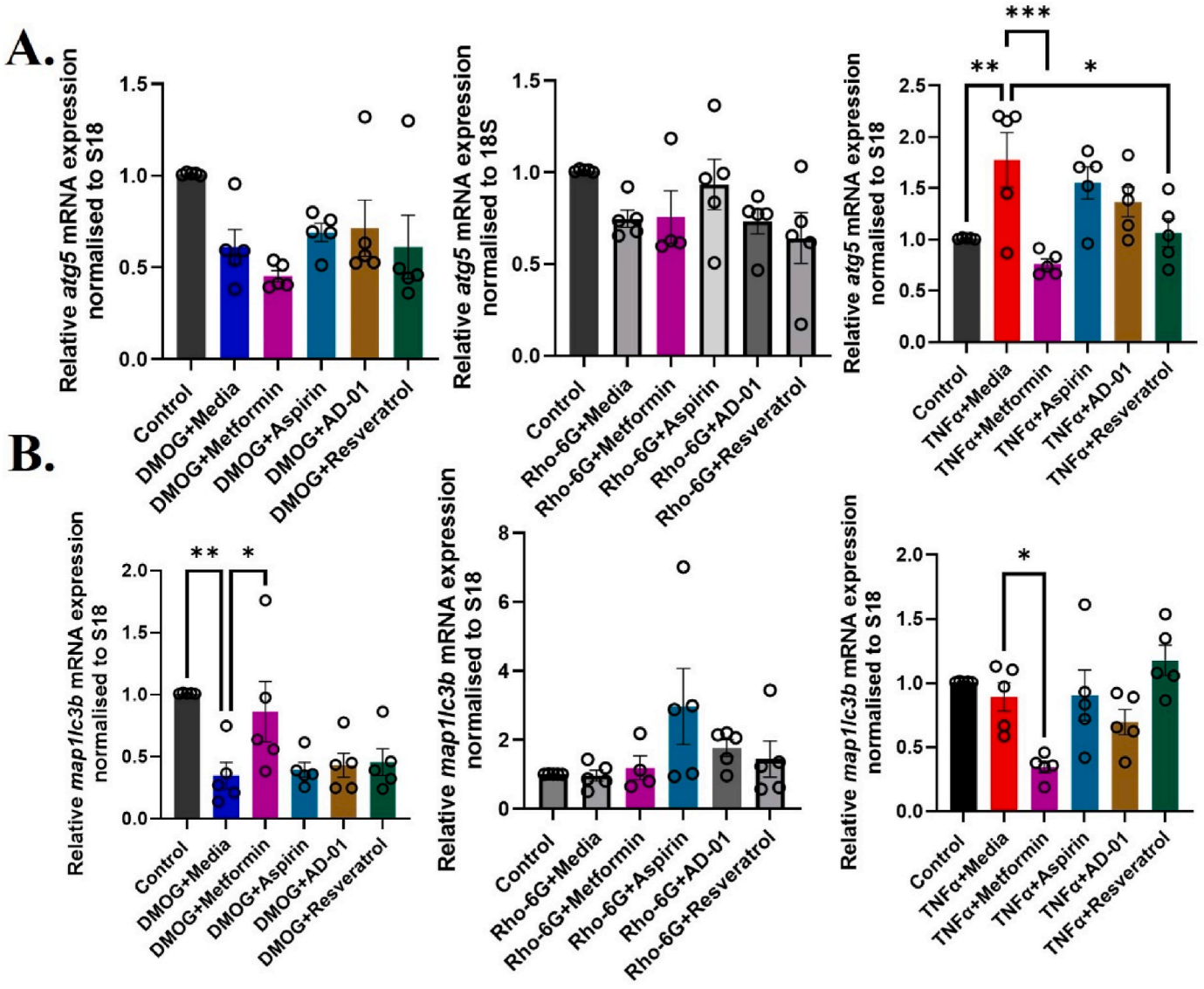

Fig. 4. TNF-α upregulates mitochondrial autophagy genes, whereas HIF-1α stabilization downregulates macroautophagy gene expression; both effects are normalized by metformin. ACH-3P cells were treated for 48 h with DMOG (100 μM; hypoxia), Rho-6G (1 μg/ml; mitochondrial dysfunction) or TNF-α (10 ng/ml; inflammation) with or without metformin (0.5 mM), aspirin (0.5 mM), AD-01 (100 nM) or resveratrol (15 μM). Cells treated in culture media were used as controls. The expression of **(A)** *atg5* and **(B)** *map1lc3b* mRNA was normalised to ribosomal protein S18. Data were normally distributed and analysed by ordinary one-way ANOVA with Sidak's post-hoc test. Data are presented as mean ± SEM (n = 5 biological replicates); *p < 0.05, **p < 0.01, ***p < 0.001.

Astragaloside IV similarly engages DUSP1–PHB2 signaling to coordinate mitochondrial quality control and endoplasmic reticulum (ER)-autophagy in septic myocardial injury [61]. In coronary microvascular disease models, Tanshinone IIA acts through a Sirt5–METTL3 node to regulate the mitochondrial–ER unfolded protein response [62], and Zishen Huoxue decoction activates a Sirt5–β-tubulin axis that couples mitophagy with UPRmt to limit ischemic myocardial damage [63]. A lipid–selenium conjugate drug that accumulates in mitochondria depletes malate/fumarate and induces mitophagy-mediated suppression of necroptosis [64]. Together, these studies highlight PHB2, Sirt5-dependent UPR/mitophagy pathways and TCA-cycle reprogramming as tractable mitochondrial targets in cardiovascular injury, providing potential mechanistic pathways for our strategy of targeting trophoblast mitochondrial quality control in preeclampsia.

The fluorescence imaging results reveal that exposure to preeclamptic stimuli causes morphological changes to the mitochondrial network, and each type of preeclamptic stimulus (HIF-1α activation, inflammation or mitochondrial dysfunction) yields a unique mitochondrial organization within the cells. Preeclampsia-like stimuli reduced mitochondrial size leading to varying degrees of aggregation, highlighting how different stresses lead to both morphological and metabolic changes in placental trophoblasts. In this study, cells were cultured in serum-reduced media prior to the addition of treatments to partially synchronize them in the same cell-cycle phase and minimize background variability in metabolic activity. This approach ensured that any observed changes could be more confidently attributed to the applied stimuli rather than differences in growth stage or serum-driven signaling. Previous work has shown that mitochondrial morphology is important for maintaining the form and function of cells [65,66]. These findings align with our previous work showing that these stressors impair trophoblast proliferation and migration [38] that could lead to preeclampsia development [67–69]. Overall, metformin treatment appeared the most effective at protecting against TNF-α-induced mitochondrial network disorganization. This morphological protection was consistent with our Seahorse XF Mito Stress Test results, where metformin significantly improved basal respiration, ATP production,

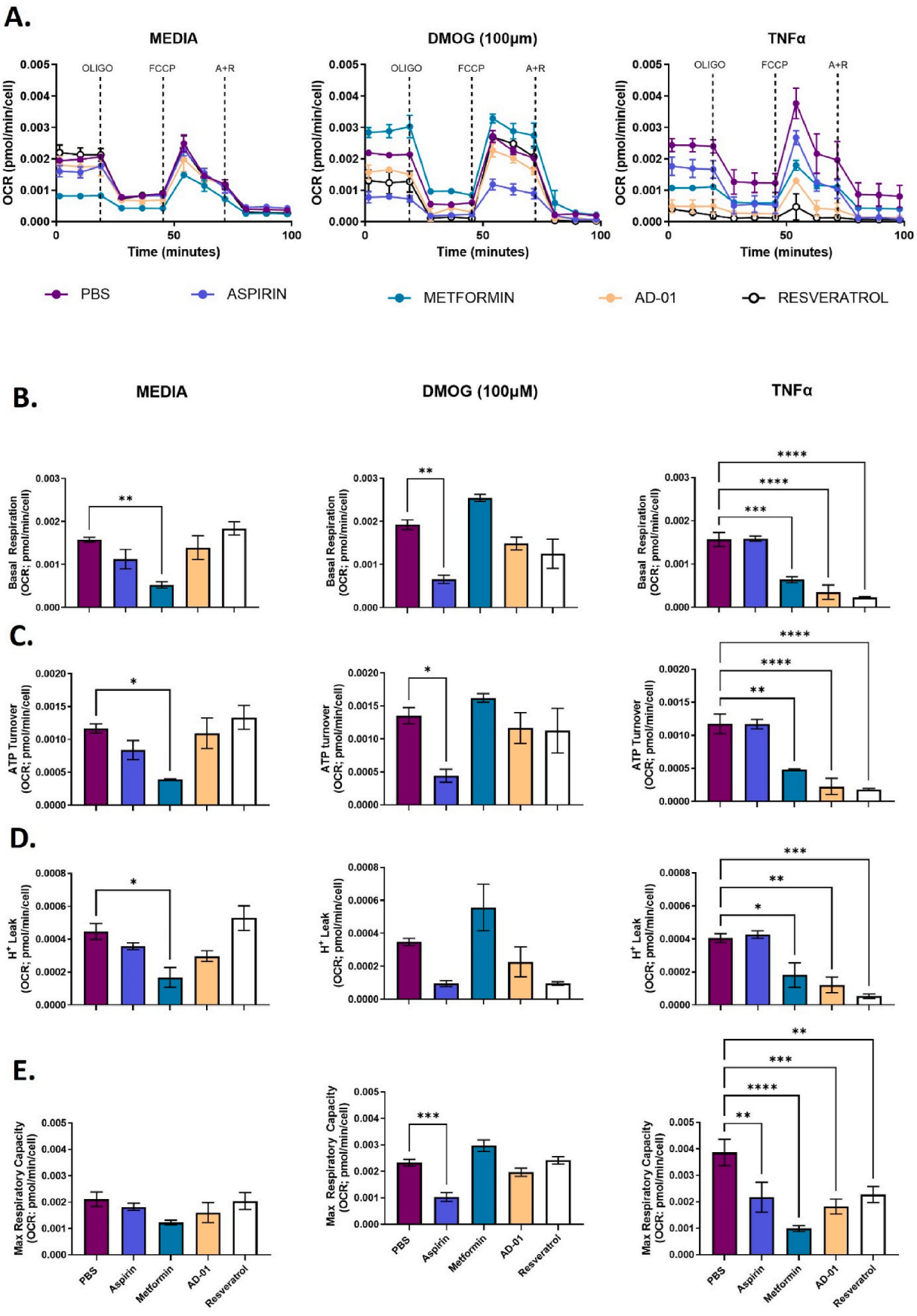

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Fig. 5. HIF-1α activation reflective of hypoxia or TNF-α inflammatory stress impact on trophoblast mitochondrial respiration; aspirin protects against HIF-1α-induced mitochondrial dysfunction, whereas metformin, AD-01 or resveratrol provide protection in inflammatory conditions. (A) Normalized OCR (pmol/min/cell) during the Mito Stress Test for ACH-3P cells treated with PBS, DMOG (100 μM), or TNF-α (10 ng/ml), in the presence of metformin (0.5 mM), aspirin (0.5 mM), AD-01 (100 nM), or resveratrol (15 μM) for 48 h. The traces illustrate the effects of sequential inhibitor additions on OCR and the bio-energetic parameters derived from these assays under each stimulus and treatment. (B–E) Bar graphs showing individual data points for **(B)** basal Respiration, **(C)** ATP Turnover, **(D)** H^+ (proton) Leak and **(E)** maximal respiratory capacity, Data were normally distributed and analysed by ordinary one-way ANOVA with Tukey's post-hoc test. Data are presented as mean ± SEM (n = 3 biological repeats); *p < 0.05 and **p < 0.01. *Key: OLIGO (Oligomycin); A + R (Antimycin A & Rotenone).*

protein leak and maximal respiration capacity in TNF-α-treated cells, indicating that its structural rescue was accompanied by functional restoration of mitochondrial bioenergetics. While proton leak reduction can indicate improved coupling efficiency, it may also reflect diminished mitochondrial adaptability and thus should not be interpreted as inherently beneficial in the context of preeclampsia. A study in a mouse model for Parkinson's disease reported that metformin can normalize mitochondrial function and reduce senescence in astrocytes [58].

Mitochondria maintain homeostasis under stress through dynamic morphological changes involving two key processes: fusion and fission [25,52,67]. Our results demonstrate that mainly fusion events, but not fission events, are impacted by preeclampsia-like conditions at the cellular level. This supports our microscopy observations showing altered structure and breakdown of the mitochondrial network. The fusion gene, *mfn1,* was downregulated under hypoxic or mitochondrial dysfunction conditions, whereas it was upregulated under inflammatory conditions, and metformin reversed this increase. The mechanism is likely via the AMPK and PGC-1α signaling, given metformin is known to signal through these pathways [70–72]. This finding aligns with a report in DU145 prostate cancer cells showing that metformin led to a decreased expression of another mitochondrial fusion protein, *mfn2* [68]. We acknowledge that further exploration of additional fission and fusion genes, including *fis1* and *opa1*, could provide better insight into mitochondrial dynamics in preeclampsia [65]. In support of this, NR4A1-driven myocardial ischemia–reperfusion injury has been shown to depend on mitochondrial fission factor (MFF)–mediated mitochondrial fragmentation together with inhibition of FUNDC1-dependent mitophagy [73], and microtubule remodelling can profoundly alter mitochondrial trafficking and bioenergetics in cardiovascular disease models [74]. These data reinforce the need to interrogate a broader panel of fission/fusion regulators and cytoskeletal interactions in trophoblasts to fully define how preeclampsia-related stresses re-shape mitochondrial networks. Related to autophagy, we found that only the inflammatory stimulus increased *atg5* expression and that both metformin and resveratrol restored its expression to normal. Autophagy activation has been previously observed in preeclampsia within placental tissue [75]. Notably, fine-tuning mitophagy appears to be context dependent: while mitophagy induction by lipid–selenium conjugates can suppress necroptosis in cancer models [64], cardioprotective agents such as Astragaloside IV harness PHB2-mediated mitochondrial quality control and ER-autophagy to limit septic myocardial injury [61]. Together with our data on *atg5* and *map1lc3b*, these findings suggest that targeted modulation of mitophagy and PHB2-linked surveillance, rather than global autophagy activation, may be required to achieve trophoblast protection in preeclampsia.

Pro-inflammatory TNF-α impaired mitochondrial respiration. These findings align with the morphological observations and suggest that structural disorganization of mitochondria is closely linked to functional impairment [70]. All baseline ACH-3P cultures were maintained under atmospheric oxygen (~21 % O_2) prior to treatment, ensuring that hypoxia-related responses in DMOG-treated groups were mediated via HIF-1α rather than differences in background oxygen tension. Consistent with other experiments, metformin was the most effective at protecting mitochondrial function, particularly in response to TNF-α-induced inflammation. Metformin treatment decreases basal respiration, ATP turnover and maximal respiration capacity, indicating its potential to counteract inflammation-induced mitochondrial dysfunction [76]. It is important to note, however, that while metformin, AD-01 and resveratrol reduced basal respiration, proton leak, ATP production and maximal respiratory capacity, these changes should not automatically be interpreted as beneficial. Such reductions could reflect improved coupling efficiency but might also signal diminished mitochondrial adaptability or energy production capacity, particularly in the context of placental insufficiency. Further functional studies are required to determine whether these bioenergetic shifts support or impair trophoblast and placental growth in preeclampsia. However, these observations are consistent with previous reports that metformin can normalize mitochondrial function and reduce cellular senescence in various disease models [77–79]. They also align with broader work showing that natural products and phytochemicals can modulate mitochondrial ROS, mitophagy and ER stress to restore redox balance in lung and cardiovascular disorders [80,81], and with comprehensive reviews highlighting mitochondrial dysfunction, impaired mitophagy and inappropriate biogenesis as central drivers of vascular endothelial injury across cardiovascular, renal and pulmonary diseases [82,83]. Given that preeclampsia combines placental mitochondrial stress with systemic endothelial dysfunction, our demonstration that metformin, resveratrol and AD-01 modulate trophoblast mitochondrial function provides a mechanistic basis for future strategies that co-target placental and maternal vascular mitochondria.

The clinical implications of using metformin, resveratrol, and AD-01 to target mitochondrial dysfunction in preeclampsia are significant. Metformin has been used to treat gestational diabetes in pregnancy for decades [84], and emerging clinical evidence supports its therapeutic potential in preeclampsia [46]. However, further clinical studies are needed to determine its short- and long-term safety and optimal dosing during pregnancy. Future studies should establish the impact on fetal health and evaluate its effectiveness in the clinical trial setting for different phenotypes of preeclampsia.

Similarly, resveratrol, a naturally occurring antioxidant, exhibits mitochondrial protective effects by inhibiting oxidative stress pathways. Despite its potential, concerns remain regarding its ability to cross the placenta and its long-term impact on fetal development. AD-01, a novel FKBPL-based peptide, demonstrated promising effects on mitochondrial integrity in this study and can restore angiogenic balance [31], but its safety and pharmacokinetics in pregnancy require further investigation *in vivo* before clinical use. In parallel, lifestyle-related chronic metabolic and inflammatory stress has been implicated in progressive mitochondrial dysfunction and electrical remodelling in sick sinus syndrome [85], underscoring that systemic mitochondrial vulnerability may modify cardiovascular risk over the life course. Integrating such systemic risk factors with placental mitochondrial targets may help refine which women are most likely to benefit from mitochondria-directed therapies.

Other mitochondrial-targeted therapies, such as melatonin and CoQ10, have shown efficacy in enhancing mitochondrial biogenesis and reducing oxidative stress in preeclampsia models. Melatonin's antioxidant properties help scavenge free radicals, reduce lipid peroxidation and protect mitochondrial integrity [86]. Similarly, CoQ10, a component of the electron transport chain, supports mitochondrial biogenesis and reduces oxidative damage by enhancing ATP production and stabilizing cellular energy levels [87]. The therapies evaluated in this study, though different from melatonin and CoQ10 in their primary modes of action, converge in their ability to restore mitochondrial function and reduce oxidative stress [38].

Finally, our *in vitro* model closely recapitulates the detrimental impact of preeclampsia-like stresses on mitochondrial morphology,

function and oxidative stress in trophoblast cells [38], providing a reliable platform for investigating mitochondria-targeting therapeutic strategies.

Despite the valuable insights, our study has several limitations, including the use of the ACH-3P trophoblast cell line. ACH-3Ps cells, although widely used and resembling first-trimester primary trophoblasts in integrin and MMP expression as well as in transcriptomics profile [39], have an abnormal karyotype (94–98 chromosomes), are derived from male tissue, so nuclear–mitochondrial interactions may differ from those in primary cells. However, our previous study validated our model against human clinical samples from women with preeclampsia showing aligned oxidative stress profile [38]. They do not fully replicate the *in vivo* placental microenvironment or enable syncytialization in 2D culture, although express β-hCG [36]. In addition, the azaserine–hypoxanthine selection medium used to maintain ACH-3Ps is removed well before experiments, but this culture history could still influence mitochondrial physiology. The final DMSO concentration in all assays was $\leq$0.1 % (v/v), which we confirmed in preliminary tests to have no measurable impact on our readouts; hence, media-only controls were deemed sufficient for subsequent assays. Future validation in primary human trophoblasts, placental organoids and ex-vivo perfusion models will be essential to confirm our findings. Moreover, mitochondrial analysis using confocal microscopy is limited by algorithms unable to detect clustered or closely spaced mitochondria, leading to underestimation and potential bias. Advanced imaging methods-such as STED, SIM, Airyscan and live-cell fluorescent tracking could enhance assessment of mitochondrial dynamics. Further validation of our findings, including metformin's therapeutic potential, should be carried out in animal models and clinical studies. Future integration of organoid models, co-culture systems and *ex-vivo* placental perfusion models may help bridge the translational gap and support targeting mitochondrial dysfunction as a therapeutic strategy for preeclampsia.

These findings also have potential implications when considering the heterogeneity of preeclampsia. Early-onset preeclampsia is often associated with placental dysfunction, and therefore might be more sensitive to interventions that improve mitochondrial biogenesis and function, whereas late-onset disease may benefit more from therapies targeting maternal endothelial dysfunction and inflammation [88]. Our *in vitro* model more closely resembles the placental pathophysiology of early-onset disease, given the emphasis on trophoblast mitochondrial impairment under hypoxic and inflammatory stress. In addition, comparison with other promising mitochondrial-targeted or antioxidant therapies, such as sulforaphane, is warranted. Sulforaphane, a naturally occurring isothiocyanate, has been shown to activate the Nrf2 pathway, reduce oxidative stress, and improve mitochondrial function in various models, including pregnancy complications [89]. While its mechanisms differ from those of metformin, resveratrol, and AD-01, these agents share a common goal of restoring mitochondrial homeostasis and reducing oxidative injury. Future studies should evaluate how these therapeutic strategies perform side-by-side in both early- and late-onset preeclampsia models to inform tailored interventions.

Although this study provides important mechanistic insights, several constraints must be addressed before our findings can be translated into clinical practice. Firstly, our work is based on a single trophoblast cell line and does not incorporate the maternal vascular endothelium, immune cells or haemodynamic factors, even though mitochondrial dysfunction in endothelial cells is now recognised as a central driver of vascular injury across cardiovascular, renal and pulmonary diseases [82,83]. Secondly, we did not assess pharmacokinetics, placental transfer, or maternal–fetal safety for metformin, resveratrol, or AD-01, all of which will be critical for determining feasible dosing windows and off-target effects in pregnancy. Interestingly, aspirin is already used clinically for prevention of preterm preeclampsia however it showed limited mitochondria-targeting therapeutic potential in our study. Finally, the study focused on mitochondrial structure, dynamics and respiration but did not evaluate downstream functional outcomes such as spiral artery remodelling, endocrine signaling, or long-term vascular programming in the offspring. Addressing these gaps in primary trophoblasts, advanced placental models and relevant *in vivo* systems will be essential to establish whether mitochondria-targeted interventions can realistically be developed as preventive or therapeutic strategies for preeclampsia.

5. Conclusions

In our ACH-3P trophoblast model, hypoxia and inflammation disrupted mitochondrial networks and dynamics, supporting a role for mitochondrial dysfunction in the pathophysiology of preeclampsia. Among the tested interventions, metformin improved aspects of mitochondrial architecture and normalized the expression of genes involved in fusion and autophagy under inflammatory conditions, with associated improvements in bioenergetic parameters. Resveratrol and AD-01 exerted modest, context-dependent effects. AD-01 partially preserved mitochondrial respiratory integrity under inflammatory conditions by reducing excessive basal respiration and oxygen consumption, although it did not substantially restore mitochondrial morphology or number. Aspirin had limited efficacy, showing some benefit under hypoxic conditions with no impact on inflammation-induced dysfunction.

These variable responses highlight the complexity of mitochondrial adaptation to different stressors and suggest that targeted interventions may need to be tailored to specific pathogenic characteristics of preeclampsia. Given this was an *in vitro* study, further work in primary cells, organoids, and *in vivo* models is needed to confirm these findings, evaluate protein-level changes and assess safety. Future research should optimize dosing and explore precision-medicine strategies with the aim of improving maternal and fetal outcomes in preeclampsia.

CRediT authorship contribution statement

Dinara Afrose: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Sofía Alfonso-Sánchez:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Ashleigh Philp:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Philip M. Hansbro:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Qian Peter Su:** Writing – review & editing, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **Lana McClements:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Conceptualization.

Ethics approval and consent to participate

Ethical approval for this project was obtained from the University of Technology Sydney (UTS).

Consent for publication

All the authors have consented to the publication in the Placenta journal.

Availability of data and materials

All the data generated or analysed during this study are available from the corresponding author upon resealable request.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbi.2025.111883.

List of Abbreviations

ACH-3P	Antiproliferative cytotrophoblast cell line-3P
AD-01	FKBPL-derived therapeutic peptide
ATP	Adenosine triphosphate
BSA	Bovine serum albumin
DMOG	Dimethyloxalylglycine
ETC	Electron transport chain
FKBPL	FK506-binding protein like
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
HIF-1α	Hypoxia-inducible factor 1-alpha
mtROS	Mitochondrial reactive oxygen species
OCR	Oxygen consumption rate
qPCR	Quantitative polymerase chain reaction
ROS	Reactive oxygen species
RT-qPCR	Reverse transcription quantitative PCR

Data availability

Data will be made available on request.

References

[1] S. Rana, E. Lemoine, J.P. Granger, S.A. Karumanchi, Preeclampsia, Circ. Res. 124 (7) (Mar. 2019) 1094–1112, https://doi.org/10.1161/CIRCRESAHA.118.313276.
[2] C.W. Ives, R. Sinkey, I. Rajapreyar, A.T.N. Tita, S. Oparil, Preeclampsia—pathophysiology and clinical presentations, J. Am. Coll. Cardiol. 76 (14) (Oct. 2020) 1690–1702, https://doi.org/10.1016/j.jacc.2020.08.014.
[3] B. Sibai, G. Dekker, M. Kupferminc, Pre-eclampsia, Lancet 365 (9461) (Feb. 2005) 785–799, https://doi.org/10.1016/S0140-6736(05)17987-2.
[4] A.L. Tranquilli, et al., The classification, diagnosis and management of the hypertensive disorders of pregnancy: a revised statement from the ISSHP, Pregnancy Hypertens. An Int. J. Women's Cardiovasc. Heal. 4 (2) (Apr. 2014) 97–104, https://doi.org/10.1016/j.preghy.2014.02.001.
[5] P. Lopez-Jaramillo, J. Barajas, S.M. Rueda-Quijano, C. Lopez-Lopez, C. Felix, Obesity and preeclampsia: common pathophysiological mechanisms, Front. Physiol. 9 (Dec. 2018), https://doi.org/10.3389/fphys.2018.01838.
[6] E. Dimitriadis, et al., Pre-eclampsia, Nat. Rev. Dis. Primers 9 (1) (Feb. 2023) 8, https://doi.org/10.1038/s41572-023-00417-6.
[7] X.-Q. Hu, L. Zhang, Mitochondrial dysfunction in the pathogenesis of preeclampsia, Curr. Hypertens. Rep. 24 (6) (Jun. 2022) 157–172, https://doi.org/10.1007/s11906-022-01184-7.
[8] P. Vangrieken, et al., Placental mitochondrial abnormalities in preeclampsia, Reprod. Sci. 28 (8) (Aug. 2021) 2186–2199, https://doi.org/10.1007/s43032-021-00464-y.
[9] J.J. Fisher, L.A. Bartho, A.V. Perkins, O.J. Holland, Placental mitochondria and reactive oxygen species in the physiology and pathophysiology of pregnancy, Clin. Exp. Pharmacol. Physiol. 47 (1) (Jan. 2020) 176–184, https://doi.org/10.1111/1440-1681.13172.
[10] A.N. Smith, X. Wang, D.G. Thomas, R.E. Tatum, G.W. Booz, M.W. Cunningham, The role of mitochondrial dysfunction in preeclampsia: causative factor or collateral damage? Am. J. Hypertens. 34 (5) (May 2021) 442–452, https://doi.org/10.1093/ajh/hpab003.
[11] P. Vangrieken, et al., Hypoxia-induced mitochondrial abnormalities in cells of the placenta, PLoS One 16 (1) (Jan. 2021) e0245155, https://doi.org/10.1371/journal.pone.0245155.
[12] A.N. Sferruzzi-Perri, J.S. Higgins, O.R. Vaughan, A.J. Murray, A.L. Fowden, Placental mitochondria adapt developmentally and in response to hypoxia to support fetal growth, Proc. Natl. Acad. Sci. 116 (5) (Jan. 2019) 1621–1626, https://doi.org/10.1073/pnas.1816056116.
[13] Y. Yang, et al., The potent antioxidant MitoQ protects against Preeclampsia during late gestation but increases the risk of Preeclampsia when administered in early pregnancy, Antioxid. Redox Signal. 34 (2) (Jan. 2021) 118–136, https://doi.org/10.1089/ars.2019.7891.
[14] M. Lu, A.N. Sferruzzi-Perri, Placental mitochondrial function in response to gestational exposures, Placenta 104 (Jan. 2021) 124–137, https://doi.org/10.1016/j.placenta.2020.11.012.
[15] A. Olszewska, A. Szewczyk, Mitochondria as a pharmacological target: magnum overview, IUBMB Life 65 (3) (Mar. 2013) 273–281, https://doi.org/10.1002/iub.1147.
[16] A.M. Rodríguez-Cano, C.C. Calzada-Mendoza, G. Estrada-Gutierrez, J.A. Mendoza-Ortega, O. Perichart-Perera, Nutrients, mitochondrial function, and perinatal health, Nutrients 12 (7) (Jul. 2020) 2166, https://doi.org/10.3390/nu12072166.
[17] M.S. Patel, N.S. Nemeria, W. Furey, F. Jordan, The pyruvate dehydrogenase complexes: structure-based function and regulation, J. Biol. Chem. 289 (24) (Jun. 2014) 16615–16623, https://doi.org/10.1074/jbc.R114.563148.
[18] A.J. Kuszak, et al., Nutritional interventions for mitochondrial OXPHOS deficiencies: mechanisms and model systems, Annu. Rev. Pathol. Mech. Dis. 13 (1) (Jan. 2018) 163–191, https://doi.org/10.1146/annurev-pathol-020117-043644.
[19] P.S. Ramsey, J.R. Biggio, Carnitine palmitoyltransferase deficiency in pregnancy, J. Matern. Neonatal Med. 18 (5) (Nov. 2005) 357–359, https://doi.org/10.1080/14767050500275861.
[20] R. Marín, D.I. Chiarello, C. Abad, D. Rojas, F. Toledo, L. Sobrevia, Oxidative stress and mitochondrial dysfunction in early-onset and late-onset preeclampsia, Biochim. Biophys. Acta - Mol. Basis Dis. 1866 (12) (Dec. 2020) 165961, https://doi.org/10.1016/j.bbadis.2020.165961.
[21] C. Mammucari, R. Rizzuto, Signaling pathways in mitochondrial dysfunction and aging, Mech. Ageing Dev. 131 (7–8) (Jul. 2010) 536–543, https://doi.org/10.1016/j.mad.2010.07.003.
[22] A. Nakashima, et al., Current understanding of autophagy in pregnancy, Int. J. Mol. Sci. 20 (9) (May 2019) 2342, https://doi.org/10.3390/ijms20092342.
[23] S. Bezerra Maia e Holanda Moura, L. Marques Lopes, P. Murthi, F. da Silva Costa, Prevention of Preeclampsia, J. Pregnancy 2012 (2012) 1–9, https://doi.org/10.1155/2012/435090.
[24] M.H. Davenport, et al., Prenatal exercise for the prevention of gestational diabetes mellitus and hypertensive disorders of pregnancy: a systematic review and meta-analysis, Br. J. Sports Med. 52 (21) (Nov. 2018) 1367–1375, https://doi.org/10.1136/bjsports-2018-099355.
[25] L. Sanchez-Aranguren, S. Nadeem, Bioenergetics adaptations and redox homeostasis in pregnancy and related disorders, Mol. Cell. Biochem. 476 (11) (Nov. 2021) 4003–4018, https://doi.org/10.1007/s11010-021-04215-0.
[26] F. Jahan, G. Vasam, A.E. Green, S.A. Bainbridge, K.J. Menzies, Placental mitochondrial function and dysfunction in Preeclampsia, Int. J. Mol. Sci. 24 (4) (Feb. 2023) 4177, https://doi.org/10.3390/ijms24044177.
[27] D.L. Rolnik, et al., Aspirin versus placebo in pregnancies at high risk for Preterm Preeclampsia, N. Engl. J. Med. 377 (7) (Aug. 2017) 613–622, https://doi.org/10.1056/NEJMoa1704559.
[28] E. Bujold, et al., Prevention of Preeclampsia and intrauterine growth restriction with aspirin started in early pregnancy, Obstet. Gynecol. 116 (2) (Aug. 2010) 402–414, https://doi.org/10.1097/AOG.0b013e3181e9322a.
[29] L. Duley, D.J. Henderson-Smart, S. Meher, J.F. King, Antiplatelet agents for preventing pre-eclampsia and its complications, Cochrane Database Syst. Rev. (Apr. 2007), https://doi.org/10.1002/14651858.CD004659.pub2.
[30] A. Yakkundi, et al., The anti-migratory effects of FKBPL and its peptide derivative, AD-01: regulation of CD44 and the cytoskeletal pathway, PLoS One 8 (2) (Feb. 2013) e55075, https://doi.org/10.1371/journal.pone.0055075.
[31] L. McClements, et al., Targeting treatment-resistant breast cancer stem cells with FKBPL and its peptide derivative, AD-01, via the CD44 pathway, Clin. Cancer Res. 19 (14) (Jul. 2013) 3881–3893, https://doi.org/10.1158/1078-0432.CCR-13-0595.
[32] S. Annett, et al., FKBPL-based peptide, ALM201, targets angiogenesis and cancer stem cells in ovarian cancer, Br. J. Cancer 122 (3) (Feb. 2020) 361–371, https://doi.org/10.1038/s41416-019-0649-5.
[33] S. Ghorbanpour, et al., The FKBPL-based therapeutic peptide, AD-01, protects the endothelium from hypoxia-induced damage by stabilising hypoxia inducible factor-α and inflammation, J. Transl. Med. 23 (1) (Mar. 2025) 309, https://doi.org/10.1186/s12967-025-06118-w.
[34] M. Chhor, et al., FK506-Binding protein like (FKBPL) has an important role in heart failure with preserved ejection fraction pathogenesis with potential diagnostic utility, Biomolecules 13 (2) (Feb. 2023) 395, https://doi.org/10.3390/biom13020395.
[35] N. Todd, et al., Role of A novel angiogenesis FKBPL-CD44 pathway in Preeclampsia risk stratification and mesenchymal stem cell treatment, J. Clin. Endocrinol. Metab. 106 (1) (Jan. 2021) 26–41, https://doi.org/10.1210/clinem/dgaa403.
[36] S.M. Ghorbanpour, et al., A placenta-on-a-chip model to determine the regulation of FKBPL and galectin-3 in preeclampsia, Cell. Mol. Life Sci. 80 (2) (Feb. 2023) 44, https://doi.org/10.1007/s00018-022-04648-w.

[37] A. Yakkundi, et al., FKBPL is a critical antiangiogenic regulator of developmental and pathological angiogenesis, Arterioscler. Thromb. Vasc. Biol. 35 (4) (Apr. 2015) 845–854, https://doi.org/10.1161/ATVBAHA.114.304539.
[38] D. Afrose, et al., Evaluating oxidative stress targeting treatments in in vitro models of placental stress relevant to preeclampsia, Front. Cell Dev. Biol. 13 (Feb) (2025), https://doi.org/10.3389/fcell.2025.1539496.
[39] U. Hiden, M. Bilban, M. Knöfler, G. Desoye, Kisspeptins and the placenta: regulation of trophoblast invasion, Rev. Endocr. Metab. Disord. 8 (1) (Mar. 2007) 31–39, https://doi.org/10.1007/s11154-007-9030-8.
[40] A. Singh, J.W. Wilson, C.J. Schofield, R. Chen, Hypoxia-inducible factor (HIF) prolyl hydroxylase inhibitors induce autophagy and have a protective effect in an in-vitro ischaemia model, Sci. Rep. 10 (1) (Jan. 2020) 1597, https://doi.org/10.1038/s41598-020-58482-w.
[41] F.C. Brownfoot, et al., Combining metformin and sulfasalazine additively reduces the secretion of antiangiogenic factors from the placenta: implications for the treatment of preeclampsia, Placenta 95 (Jun. 2020) 78–83, https://doi.org/10.1016/j.placenta.2020.04.010.
[42] Z. Wang, et al., Acetylcholine ameliorated TNF-α-induced primary trophoblast malfunction via muscarinic receptors, Biol. Reprod. 103 (6) (Dec. 2020) 1238–1248, https://doi.org/10.1093/biolre/ioaa158.
[43] J. Dutra Silva, et al., Mesenchymal stromal cell extracellular vesicles rescue mitochondrial dysfunction and improve barrier integrity in clinically relevant models of ARDS, Eur. Respir. J. 58 (1) (Jul. 2021) 2002978, https://doi.org/10.1183/13993003.02978-2020.
[44] A.R. Gear, Rhodamine 6G. A potent inhibitor of mitochondrial oxidative phosphorylation, J. Biol. Chem. 249 (11) (Jun. 1974) 3628–3637.
[45] A.R.L. Gear, Rhodamine 6G, J. Biol. Chem. 249 (11) (Jun. 1974) 3628–3637, https://doi.org/10.1016/S0021-9258(19)42620-3.
[46] C.A. Cluver, et al., Use of metformin to prolong gestation in preterm pre-eclampsia: randomised, double blind, placebo controlled trial, BMJ (Sep. 2021) n2103, https://doi.org/10.1136/bmj.n2103.
[47] C.S. Han, et al., Glucose and metformin modulate human first trimester trophoblast function: a model and potential therapy for diabetes-associated uteroplacental insufficiency, Am. J. Reprod. Immunol. 73 (4) (Apr. 2015) 362–371, https://doi.org/10.1111/aji.12339.
[48] L. McClements, et al., FKBPL and its peptide derivatives inhibit endocrine therapy resistant cancer stem cells and breast cancer metastasis by downregulating DLL4 and Notch4, BMC Cancer 19 (1) (Dec. 2019) 351, https://doi.org/10.1186/s12885-019-5500-0.
[49] Z. Shi, W. Long, C. Zhao, X. Guo, R. Shen, H. Ding, Comparative proteomics analysis suggests that placental mitochondria are involved in the development of pre-eclampsia, PLoS One 8 (5) (May 2013) e64351, https://doi.org/10.1371/journal.pone.0064351.
[50] S. Viana-Mattioli, et al., SIRT1-dependent effects of resveratrol and grape juice in an in vitro model of preeclampsia, Biomed. Pharmacother. 131 (Nov. 2020) 110659, https://doi.org/10.1016/j.biopha.2020.110659.
[51] J. Ding, Y. Kang, Y. Fan, Q. Chen, Efficacy of resveratrol to supplement oral nifedipine treatment in pregnancy-induced preeclampsia, Endocr. Connect. 6 (8) (Nov. 2017) 595–600, https://doi.org/10.1530/EC-17-0130.
[52] A El Helali, et al., A first-in-human Phase I dose-escalation trial of the novel therapeutic peptide, ALM201, demonstrates a favourable safety profile in unselected patients with ovarian cancer and other advanced solid tumours, Br J Cancer 127 (1) (2022) 92–101, https://doi.org/10.1038/s41416-022-01780-z.
[53] L. Gong, S. Goswami, K.M. Giacomini, R.B. Altman, T.E. Klein, Metformin pathways, Pharmacogenet. Genomics 22 (11) (Nov. 2012) 820–827, https://doi.org/10.1097/FPC.0b013e3283559b22.
[54] D.J. Angiolillo, et al., Pharmacokinetic and pharmacodynamic profile of a novel phospholipid aspirin formulation, Clin. Pharmacokinet. 61 (4) (Apr. 2022) 465–479, https://doi.org/10.1007/s40262-021-01090-2.
[55] A. Nakashima, et al., Disruption of placental homeostasis leads to Preeclampsia, Int. J. Mol. Sci. 21 (9) (May 2020) 3298, https://doi.org/10.3390/ijms21093298.
[56] J.J. Fisher, et al., Mitochondrial dysfunction in placental trophoblast cells experiencing gestational diabetes mellitus, J. Physiol. 599 (4) (Feb. 2021) 1291–1305, https://doi.org/10.1113/JP280593.
[57] A. Maloyan, J. Mele, B. Muralimanohara, L. Myatt, Measurement of mitochondrial respiration in trophoblast culture, Placenta 33 (5) (May 2012) 456–458, https://doi.org/10.1016/j.placenta.2012.01.016.
[58] Y. Wu, et al., Mitochondrial quality control alterations and placenta-related disorders, Front. Physiol. 15 (Feb) (2024), https://doi.org/10.3389/fphys.2024.1344951.
[59] V. Anesti, L. Scorrano, The relationship between mitochondrial shape and function and the cytoskeleton, Biochim. Biophys. Acta - Bioenerg. 1757 (5–6) (May 2006) 692–699, https://doi.org/10.1016/j.bbabio.2006.04.013.
[60] J. Wang, et al., Ligustrazine nano-drug delivery system ameliorates doxorubicin-mediated myocardial injury via piezo-type mechanosensitive ion channel component 1-prohibitin 2-mediated mitochondrial quality surveillance, J. Nanobiotechnology 23 (1) (May 2025) 383, https://doi.org/10.1186/s12951-025-03420-z.
[61] J. Wang, et al., Astragaloside IV alleviates septic myocardial injury through DUSP1-Prohibitin 2 mediated mitochondrial quality control and ER-autophagy, J. Adv. Res. 75 (Sep. 2025) 561–580, https://doi.org/10.1016/j.jare.2024.10.030.
[62] X. Pu, et al., Tanshinone IIA modulates Sirt5 and Metll3 interaction to govern mitochondria-endoplasmic reticulum unfolded protein response in coronary microvascular injury, Phytomedicine 145 (Sep. 2025) 156982, https://doi.org/10.1016/j.phymed.2025.156982.
[63] X. Chang, et al., Zishen Huoxue decoction (ZSHX) alleviates ischemic myocardial injury (MI) via Sirt5-β-tubulin mediated synergistic mechanism of 'mitophagy-unfolded protein response' and mitophagy, Chin. J. Nat. Med. 23 (3) (Mar. 2025) 311–321, https://doi.org/10.1016/S1875-5364(25)60838-7.
[64] X. Chang, et al., Targeting mitochondria by lipid-selenium conjugate drug results in malate/fumarate exhaustion and induces mitophagy-mediated necroptosis suppression, Int. J. Biol. Sci. 20 (14) (Oct. 2024) 5793–5811, https://doi.org/10.7150/ijbs.102424.
[65] S. Campello, L. Scorrano, Mitochondrial shape changes: orchestrating cell pathophysiology, EMBO Rep. 11 (9) (Sep. 2010) 678–684, https://doi.org/10.1038/embor.2010.115.
[66] B. Huppertz, The critical role of abnormal trophoblast development in the etiology of Preeclampsia, Curr. Pharm. Biotechnol. 19 (10) (Nov. 2018) 771–780, https://doi.org/10.2174/1389201019666180427110547.
[67] G.J. Burton, A.W. Woods, E. Jauniaux, J.C.P. Kingdom, Rheological and physiological consequences of conversion of the maternal spiral arteries for uteroplacental blood flow during human pregnancy, Placenta 30 (6) (Jun. 2009) 473–482, https://doi.org/10.1016/j.placenta.2009.02.009.
[68] Y. Zhou, et al., Reversal of gene dysregulation in cultured cytotrophoblasts reveals possible causes of preeclampsia, J. Clin. Investig. 123 (7) (Jul. 2013) 2862–2872, https://doi.org/10.1172/JCI66966.
[69] M. Wang, et al., Metformin normalizes mitochondrial function to delay astrocyte senescence in a mouse model of Parkinson's disease through Mfn2-cGAS signaling, J. Neuroinflammation 21 (1) (Apr. 2024) 81, https://doi.org/10.1186/s12974-024-03072-0.
[70] C. Loubiere, et al., The energy disruptor metformin targets mitochondrial integrity via modification of calcium flux in cancer cells, Sci. Rep. 7 (1) (Jul. 2017) 5040, https://doi.org/10.1038/s41598-017-05052-2.
[71] S.K. Nashif, et al., Metformin impairs trophoblast metabolism and differentiation in a dose-dependent manner, Front. Cell Dev. Biol. 11 (May 2023), https://doi.org/10.3389/fcell.2023.1167097.
[72] L. He, X. Wu, F. Zhan, X. Li, J. Wu, Protective role of metformin in preeclampsia via the regulation of NF-κB/sFlt-1 and Nrf2/HO-1 signaling pathways by activating AMPK, Placenta 143 (Nov. 2023) 91–99, https://doi.org/10.1016/j.placenta.2023.10.003.
[73] J. Wang, et al., Nuclear receptor subfamily 4 group A member 1 promotes myocardial ischemia/reperfusion injury through inducing mitochondrial fission factor-mediated mitochondrial fragmentation and inhibiting FUN14 domain containing 1-depedent mitophagy, Int. J. Biol. Sci. 20 (11) (2024) 4458–4475, https://doi.org/10.7150/ijbs.95853.
[74] Q. Wu, et al., Microtubules and cardiovascular diseases: insights into pathology and therapeutic strategies, Int. J. Biochem. Cell Biol. 175 (Oct. 2024) 106650, https://doi.org/10.1016/j.biocel.2024.106650.
[75] R. Akaishi, et al., Autophagy in the placenta of women with hypertensive disorders in pregnancy, Placenta 35 (12) (Dec. 2014) 974–980, https://doi.org/10.1016/j.placenta.2014.10.009.
[76] X. Liu, H. Qin, J. Xu, The role of autophagy in the pathogenesis of systemic lupus erythematosus, Int. Immunopharmacol. 40 (Nov. 2016) 351–361, https://doi.org/10.1016/j.intimp.2016.09.017.
[77] Y. Xia, R.E. Kellems, Is preeclampsia an autoimmune disease? Clin. Immunol. 133 (1) (Oct. 2009) 1–12, https://doi.org/10.1016/j.clim.2009.05.004.
[78] A.M. Bînă, et al., Impairment of mitochondrial respiration in platelets and placentas: a pilot study in preeclamptic pregnancies, Mol. Cell. Biochem. 477 (7) (Jul. 2022) 1987–2000, https://doi.org/10.1007/s11010-022-04415-2.
[79] A. Grismaldo R, L.A. Luévano-Martínez, M. Reyes, G. García-Márquez, G. García-Rivas, L. Sobrevia, Placental mitochondrial impairment and its association with maternal metabolic dysfunction, J. Physiol. (Aug. 2024), https://doi.org/10.1113/JP285935.
[80] X. Chang, et al., Potential candidates of natural antioxidants from herbs for treating lung disorders: focus on redox balance and natural products, Phyther. Res. 39 (8) (Aug. 2025) 3353–3385, https://doi.org/10.1002/ptr.70008.
[81] K. Yang, P. Zhang, J. Li, G. Zhang, X. Chang, Potential of natural drug modulation of endoplasmic reticulum stress in the treatment of myocardial injury, J. Pharm. Anal. 14 (11) (Nov. 2024) 101034, https://doi.org/10.1016/j.jpha.2024.101034.
[82] B. Pang, et al., Advances in pathogenesis and treatment of vascular endothelial injury-related diseases mediated by mitochondrial abnormality, Front. Pharmacol. 15 (Aug) (2024), https://doi.org/10.3389/fphar.2024.1422686.
[83] B. Pang, et al., Emerging insights into the pathogenesis and therapeutic strategies for vascular endothelial injury-associated diseases: focus on mitochondrial dysfunction, Angiogenesis 27 (4) (Nov. 2024) 623–639, https://doi.org/10.1007/s10456-024-09938-4.
[84] S. . W. A. Q. M. K., U.R. Jahan, Association of gestational diabetes mellitus with maternal obesity and preeclampsia: hospital-based cross-sectional study, KHYBER Med. Univ. J. 10 (1) (Mar. 2018) 19–22.
[85] X. Chang, et al., The effect of unhealthy lifestyle on the pathogenesis of sick sinus syndrome: a life-guiding review, Medicine (Baltim.) 103 (43) (Oct. 2024) e39996, https://doi.org/10.1097/MD.0000000000039996.
[86] M. Ay, et al., Mito-metformin protects against mitochondrial dysfunction and dopaminergic neuronal degeneration by activating upstream PKD1 signaling in cell culture and MitoPark animal models of Parkinson's disease, Front. Neurosci. 18 (Feb) (2024), https://doi.org/10.3389/fnins.2024.1356703.
[87] D. Sun, H. Li, Y. Du, Y. Chen, L. Yao, L. Wang, Metformin modulates mitochondrial autophagy in renal tubular epithelial injury induced by high glucose via the

Keap1/Nrf2 pathway, Mol. Cell. Biochem. (Oct. 2023), https://doi.org/10.1007/s11010-023-04843-8.

[88] G.J. Burton, C.W. Redman, J.M. Roberts, A. Moffett, Pre-eclampsia: pathophysiology and clinical implications, BMJ (Jul. 2019) l2381, https://doi.org/10.1136/bmj.l2381.

[89] A.G. Cox, et al., Sulforaphane improves endothelial function and reduces placental oxidative stress in vitro, Pregnancy Hypertens 16 (Apr. 2019) 1–10, https://doi.org/10.1016/j.preghy.2019.02.002.