IN VIVO MITOCHONDRIA-TARGETED PROTECTION AGAINST UTERINE ARTERY VASCULAR DYSFUNCTION AND REMODELING IN RODENT HYPOXIC PREGNANCY

Zhongchao Wang, MD PhD[1,2,3], Emily J Camm, PhD[1,4], Anna Maria Nuzzo, PhD[1,5], Ana-Mishel Spiroski, PhD[1,6], Katie L Skeffington, PhD[1], Thomas J Ashmore, BSc[1], Alessandro Rolfo, PhD[5], Tullia Todros, MD PhD[5], Angela Logan, PhD[6], Jin Ma, MD PhD[2], Michael P Murphy, PhD[6], *Youguo Niu, MD DPhil[1,6] & *Dino A Giussani, PhD[1,6]

[1]Department of Physiology, Development and Neuroscience, University of Cambridge, UK
[2]Department of Aerospace Physiology, Fourth Military Medical University, Xi'an, China
[3] Department of Congenital Heart Disease, General Hospital of Northern Theater Command, Shenyang, China.
[4]The Ritchie Centre, Hudson Institute of Medical Research, Australia
[5]Department of Surgical Sciences, University of Turin, Italy
[6]Cambridge Cardiovascular Strategic Research Initiative, University of Cambridge, UK
[7]MRC Mitochondrial Biology Unit, University of Cambridge, UK
**Equal contribution*

Journal: *Journal of Physiology*

Running Title: **MitoQ Protects the Uterine Circulation**

Word Count: 4078 words

Correspondence:

Dr Youguo Niu
Department of Physiology, Development and Neuroscience
University of Cambridge
Cambridge
CB2 3EG, UK
Tel: +44 1223 333894
E-mail: **dag26@cam.ac.uk**

KEY POINTS

- Dysfunction and remodelling of the uterine artery are strongly implicated in many pregnancy complications, including advanced maternal age, maternal hypertension of pregnancy, maternal obesity, gestational diabetes, and pregnancy at high altitude.

- Such complications not only have immediate adverse effects on the growth of the fetus, but they can also increase the risk of cardiovascular disease in the mother and offspring. Despite this, there is a significant unmet clinical need for therapeutics that treat uterine artery vascular dysfunction in adverse pregnancy.

- Here, we show in a rodent model of gestational hypoxia, that *in vivo* oral treatment of the mitochondria-targeted antioxidant MitoQ protects against uterine artery vascular dysfunction and remodelling, supporting the use of mitochondria-targeted therapy against adverse changes in uterine artery structure and function in high-risk pregnancy.

ABSTRACT

Gestational hypoxia adversely affects uterine artery function, increasing complications. However, effective therapy remains unidentified. Here, we show in rodent uterine arteries that hypoxic pregnancy promotes hypertrophic remodelling, increases constrictor reactivity via protein kinase C signalling, and triggers compensatory dilatation via NO-dependent mechanisms and stimulation of large conductance Ca^{2+}-activated K^+-channels. Maternal *in vivo* oral treatment with the mitochondria-targeted antioxidant MitoQ in hypoxic pregnancy normalises uterine artery reactivity and prevents vascular remodelling. From days 6-20 of gestation (term ~22 days), female Wistar rats were randomly assigned to normoxic or hypoxic (13% O_2) pregnancy +/- daily maternal MitoQ treatment (500 μM in drinking water). At 20 days of gestation, maternal, placental, and fetal tissue was frozen to determine MitoQ uptake. The uterine arteries were harvested, and in one segment, constrictor and dilator reactivity was determined by wire myography. Another segment was fixed for unbiased stereological analysis of vessel morphology. Maternal administration of MitoQ in both normoxic and hypoxic pregnancy crossed the placenta and was present in all tissues analysed. Hypoxia increased uterine artery constrictor responses to norepinephrine, angiotensin II and the protein kinase C activator PDBu. Hypoxia enhanced dilator reactivity to sodium nitroprusside, the large conductance Ca^{2+}-activated K^+-channel (BKCa) activator NS1619, and acetylcholine via increased NO-dependent mechanisms. Uterine arteries from hypoxic pregnancy showed increased wall thickness, and MitoQ treatment in hypoxic pregnancy prevented all effects on uterine artery reactivity and remodelling. The data support mitochondria-targeted therapy against adverse changes in uterine artery structure and function in high-risk pregnancy.

Key words: chronic hypoxia, preeclampsia, uterine PI, fetal growth restriction

INTRODUCTION

Pregnancy imposes a high demand on the maternal vascular system. With advancing gestation there is a significant increase in uteroplacental blood flow to sustain fetal growth (Rosenfeld, 1977; Longo, 1983; Duvekot & Peeters, 1994). Both the uterus and placenta rely on the uterine artery as the primary source of blood supply, and the uterine artery undergoes functional and structural changes during pregnancy to increase uteroplacental perfusion (Thornburg *et al.*, 2000; Mandala & Osol, 2012; Sanghavi & Rutherford, 2014; Morton *et al.*, 2017). These changes include decreased uterine constrictor and myogenic responses, increased vasodilator reactivity and outward hypertrophic growth (Weiner *et al.*, 1991; Osol & Cipolla, 1993; Kublickiene *et al.*, 1997; White *et al.*, 1998; Veerareddy *et al.*, 2002; Xiao *et al.*, 2006). Uterine artery dysfunction and remodelling are strongly implicated in clinical indications of complicated pregnancy including advanced maternal age (Pirhonen *et al.*, 2005; Care *et al.*, 2015), maternal hypertension of pregnancy (Myatt & Webster, 2009; Brennan *et al.*, 2014), maternal obesity, gestational diabetes (Stanley *et al.*, 2009; Goulopoulou *et al.*, 2014), and pregnancy at high altitude (Zamudio *et al.*, 1995b). Such complications during pregnancy have not only immediate adverse effects on the mother and fetus, but they can also increase the risk of cardiovascular disease in the adult offspring (Gluckman *et al.*, 2008; Giussani, 2021). Despite this, there is a significant unmet clinical need for therapeutics that treat uterine vascular dysfunction in adverse pregnancy.

Over 140 million people live at altitudes higher than 2500m, comprising the largest single human group at risk of maternal and fetal complications during pregnancy because of the low atmospheric oxygen availability. Data derived from human clinical studies describe altered uterine blood flow and a greater prevalence of preeclampsia in women undergoing pregnancy at high altitude (Moore *et al.*, 1982; Zamudio *et al.*, 1995a; Palmer *et al.*, 1999; Keyes *et al.*, 2003). Several preclinical mammalian models have shown that hypobaric and isobaric hypoxic pregnancy has profound effects on uterine artery remodelling and reactivity, impairing the pregnancy-induced increase in uterine perfusion, thereby increasing the risk of adverse outcomes for the mother and offspring (Hu *et al.*, 1996; Hu

& Zhang, 1997; White *et al.*, 1998; Hu *et al.*, 1999; White & Zhang, 2003). Data generated by independent laboratories support that oxidative stress may mechanistically link many of the adverse effects of hypoxic pregnancy on the mother and offspring, focusing an interest on potential antioxidant therapy (Giussani *et al.*, 2012; Richter *et al.*, 2012; Thompson & Al-Hasan, 2012; Giussani & Davidge, 2013; Kane *et al.*, 2013; Niu *et al.*, 2018; Nuzzo *et al.*, 2018; Tong & Giussani, 2019; Botting *et al.*, 2020; Camm *et al.*, 2021; Ganguly *et al.*, 2021; Giussani, 2021; Hu & Zhang, 2021; Spiroski *et al.*, 2021; Hansell *et al.*, 2022; Tong *et al.*, 2022).

Mitochondria are a major site of ROS production, therefore targeting them should be a very powerful antioxidant therapy. However, conventional antioxidants are ineffective because they cannot penetrate the mitochondria. Part of the problem relates to the difficulty of delivering antioxidants to mitochondria *in situ*. However, Murphy and colleagues (Smith & Murphy, 2010) have developed a mitochondria-targeted ubiquinone that overcomes this challenge. MitoQ is composed of a lipophilic triphenylphosphonium (TPP) cation covalently attached to an ubiquinol antioxidant. Lipophilic cations easily move through phospholipid bilayers without requiring a specific uptake mechanism. The TPP cation concentrates MitoQ several hundred-fold within mitochondria, driven by the large mitochondrial membrane potential (Cochemé *et al.*, 2007). Only within the mitochondria, MitoQ is reduced by the respiratory chain to its active ubiquinol form, which is a particularly effective antioxidant that prevents lipid peroxidation and mitochondrial damage (Smith & Murphy, 2010). The benefits of MitoQ have now been reported in a range of studies in chicken embryos, mice, rats, sheep and in Phase II human trials (See (Giussani, 2021), for review). In contrast to vitamin C and other conventional antioxidants, MitoQ has no pro-oxidant activity at high doses and chronic administration to mice and humans suggest no toxicity (Graham *et al.*, 2009; Gane *et al.*, 2010; Rodriguez-Cuenca *et al.*, 2010; Snow *et al.*, 2010; Botting *et al.*, 2020). In addition, within the oxidative stress cascade and in contrast to conventional antioxidants, MitoQ does not affect superoxide anion production but instead acts downstream of its generation by preventing lipid peroxidation

and mitochondrial damage that is initiated by superoxide (Botting *et al.*, 2020). This is important because the cellular oxidant *milieu* is also an important modulator of vascular resistance, and a main physiological function of superoxide is to readily combine with nitric oxide (NO) and contribute to vasomotion (Chen & Keaney, 2004). The relative levels of superoxide may regulate the local vascular tone, promoting vasoconstriction (increased superoxide and reduced nitric oxide levels) or vasodilation (reduced superoxide and increased nitric oxide levels) (Chen & Keaney, 2004). In the late gestation fetus, this vascular oxidant tone is physiologically active (Thakor *et al.*, 2010). During acute fetal hypoxia increased superoxide levels contributes to fetal peripheral vasoconstriction, which together with fetal carotid chemoreflex activation and fetal endocrine constrictor responses, helps to redistribute blood flow away from the periphery towards the fetal brain; the so-called fetal brain sparing response to acute hypoxia (Thakor *et al.*, 2010; Giussani, 2021). We have previously reported that MitoQ treatment in chronic hypoxic pregnancy in sheep prevents a fetal origin of cardiovascular dysfunction without affecting the fetal brain sparing to acute hypoxia (Botting *et al.*, 2020; Giussani, 2021; Spiroski *et al.*, 2021). Therefore, in contrast to conventional antioxidants, MitoQ treatment in hypoxic pregnancy prevents the adverse programming effects of hypoxic pregnancy in promoting an increased cardiovascular risk in the offspring, and it does so while maintaining the fetal capacity to redistribute blood flow away from the periphery towards the fetal brain. Whether the protective effects of MitoQ in hypoxic pregnancy transcend to protect the maternal circulation is only just beginning to be explored.

In a comprehensive and elegant series of studies in uterine arteries isolated from pregnant sheep at high altitude, Xiao and colleagues have raised the hypothesis that gestational hypoxia impairs the uterine vascular adaptation to pregnancy via an interplay between increased microRNA-210 and decreased TET methylcytosine dioxygenase 2 (TET2), which promotes enhanced mitochondria-derived ROS generation (Hu *et al.*, 2023). They showed that TET2 knockdown by siRNAs significantly decreased spontaneous transient outward currents (STOCs) and elevated myogenic tone in uterine arteries. Further, the

effect of TET2 knockdown on STOCs and myogenic tone was negated by *in vitro* treatment with MitoQ (Hu *et al.*, 2023). However, whether MitoQ is effective *in vivo* against uterine artery vascular dysfunction in hypoxic pregnancy is unknown. To further explore the therapeutic efficacy of MitoQ in adverse pregnancy, we tested the hypothesis that maternal *in vivo* oral treatment with MitoQ protects against uterine artery constrictor hyper-reactivity and vascular remodelling in an established rodent model of gestational hypoxia.

METHODS

Ethical Approval

All experiments were performed in accordance with the UK Home Office guideline under the Animals (Scientific Procedures) Act 1986 and were approved by the University of Cambridge Animal Welfare and Ethical Review Board (AWERB). Experiments were designed and reported according to the ARRIVE guidelines (Kilkenny *et al.*, 2010).

Animal Model and Experimental Design

Wistar rats (Charles River Limited, UK) were housed in individually ventilated cages (60% humidity, 21˚C, 12:12 hour light-dark cycle) with free access to food (maintenance diet, Charles River, UK) and water. Female Wistar rat pregnancies were established under standard conditions as previously described (Camm *et al.*, 2010; Herrera *et al.*, 2011; Richter *et al.*, 2012; Kane *et al.*, 2013; Allison *et al.*, 2016; Niu *et al.*, 2018; Camm *et al.*, 2021; Hansell *et al.*, 2022). Rats were randomly allocated to one of four groups (n=10-12 per group): normoxic or hypoxic pregnancy, with or without MitoQ treatment (Figure 1). MitoQ was obtained locally in a water-soluble form via our collaborators from the Mitochondrial Biology Unit at the University of Cambridge. MitoQ (500 μM) was administered in the maternal drinking water and was prepared fresh every three days, using a dose level and regimen we have previously validated to show efficacy in the same rodent model (Nuzzo *et al.*, 2018; Spiroski *et al.*, 2021). Pregnant animals allocated to hypoxic groups were housed in a hypoxic chamber, which combined a PVC isolator (PFI Plastics Ltd., U.K.) with a nitrogen generator [N2MID60, Domnick Hunter Ltd., U.K. (Camm *et al.*, 2010; Herrera *et al.*, 2011; Richter *et al.*, 2012; Kane *et al.*, 2013; Allison *et al.*, 2016; Niu *et al.*, 2018; Nuzzo *et al.*, 2018; Camm *et al.*, 2021; Spiroski *et al.*, 2021; Hansell *et al.*, 2022)]. The oxygen concentration was maintained at 13% inspired fraction of oxygen by autoregulation of nitrogen and room air at 12-20 air changes per hour, monitored continuously with an oxygen analyzer (ICA, UK). Induction of hypoxia during pregnancy prior to day 6 of gestation markedly enhances pregnancy loss (Giussani *et al.*, 2012), thus dams were exposed to hypoxia from day 6 to 20 of gestation. Hypoxia was maintained during husbandry practices by access through an isolator

transfer compartment. Daily maternal food and water intake was monitored by calculating the difference in weight of the respective receptacle before and 24h after administration, and cage changes were conducted at comparable intervals to dams maintained in normoxia. Dams allocated to normoxic groups were housed within the room containing the hypoxic isolator with a controlled 12h light/dark cycle (Camm *et al.*, 2010; Herrera *et al.*, 2011; Giussani *et al.*, 2012; Richter *et al.*, 2012; Kane *et al.*, 2013; Allison *et al.*, 2016; Niu *et al.*, 2018; Nuzzo *et al.*, 2018; Camm *et al.*, 2021; Spiroski *et al.*, 2021; Hansell *et al.*, 2022).

On day 20 of gestation, pregnant rats were weighed and killed humanely by CO_2 inhalation and cervical dislocation. The pregnant uterus was exposed via a mid-line incision and all fetuses and associated placentas were isolated and weighed. Before weighing, the umbilical cord was removed at the insertion site and the placenta and fetus were blotted on paper. The uterine artery was removed and placed into cold Krebs buffer solution consisting of (in mM): 118.3 NaCl, 4.7 KCl, 25 $NaHCO_3$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 2.5 $CaCl_2$, 11.1 glucose, and 0.026 EDTA (pH 7.4). Using a bifocal dissecting microscope (Brunel Microscopes Ltd, Chippenham, UK), the uterine artery was dissected free from surrounding connective tissues and artery segments were prepared for *ex vivo* wire myography or stereology and histological analyses. The maternal liver, placenta, fetal liver, and fetal brain were snap frozen in liquid nitrogen and stored at -80^{o}C until MitoQ tissue concentrations were quantified.

MitoQ Tissue Uptake Following *In Vivo* Oral Treatment

Maternal liver, placenta, fetal brain, and fetal liver were frozen, and samples from each tissue (50 mg) were homogenised in 300 µL of 50 mM of Tris buffer (pH 7.0), extracted with acetonitrile (Sigma-Aldrich, UK) and dried overnight under a vacuum (Savant SpeedVac). The extracts were reconstituted and the MitoQ content measured using mass spectrometry. Control tissues were spiked with known amounts of MitoQ (1-500 pmol), and the protocol was repeated to generate a standard curve. MitoQ concentration was analysed by liquid chromatography-tandem mass spectrometry (LC/MS/MS) using an I-class Acquity UPLC attached to a Xevo TQ-S triple quadruple mass spec (both Waters, Milford, USA), and expressed relative to the

internal standard by multiple reaction monitoring using the transition 583>441. The results were analysed with MassLynx software. The assay could detect as low as 0.1pmol MitoQ/100mg of tissue.

Ex Vivo Wire Myography

A 2 mm segment of the main branch of the uterine artery was mounted onto a four-chamber small-vessel wire myograph (Multi Wire Myograph System 610 M, DMT, Denmark) as previously described (Herrera *et al.*, 2011; Allison *et al.*, 2016; Skeffington *et al.*, 2016; Hansell *et al.*, 2022). The chamber was filled with Krebs buffer solution, bubbled continuously with 95% O_2, 5% CO_2 and maintained at 37°C. Vasoconstrictor responses to potassium chloride (KCl: 16-125 mmol·L^{-1}), norepinephrine (NE: 10^{-10}-10^{-4} mol·L^{-1}), angiotensin II (Ang II: 10^{-9}-10^{-5} mol·L^{-1}) and the protein kinase C (PKC) activator phorbol 12, 13-dibutyrate (PDBu: 10^{-9}-10^{-5} mol·L^{-1}) were generated. Vasodilator responses to sodium nitroprusside (SNP: 10^{-10}-10^{-4} mol·L^{-1}), acetylcholine (ACh: 10^{-9}-10^{-5} mol·L^{-1}), and the large conductance calcium activated potassium channel opener NS1619 (10^{-9}-10^{-6} mol·L^{-1}) were also determined. For relaxant responses, vessels were pre-constricted with phenylephrine (PE: 10^{-5} mol·L^{-1}). To further establish NO-dependent and NO-independent effects on endothelial function, additional concentration-dependent responses to ACh were determined following incubation with the endothelial nitric oxide synthase (eNOS) inhibitor L-NAME (10^{-5} mol·L^{-1}). The contribution of NO to the vascular relaxation induced by ACh was calculated by subtracting the area under the curve (AUC) for ACh – the AUC for ACh + LNAME. The contribution of NO-independent mechanisms to the vascular relaxation induced by ACh was calculated by the AUC for ACh + LNAME (Giussani *et al.*, 2012; Skeffington *et al.*, 2016).

Between constrictor or dilator curves, vessels were washed with Krebs solution and allowed to equilibrate for at least 20 min. Concentration-response curves were analysed using an agonist-response best-fit line. The developed tension of constrictor curves was expressed as a percentage of the maximal KCl-induced contraction. Dilator responses were expressed as percentage of the contraction induced by PE. Finally, the overall constrictor or relaxant

capacity was expressed as the AUC (Giussani *et al.*, 2012; Skeffington *et al.*, 2016).

Stereological Analysis of Vessel Morphology and Histology

Uterine artery rings (200-300 μm in diameter) were immersion fixed in 4% paraformaldehyde (PFA), embedded in paraffin wax, and sectioned at 10 μm (Leica RM 2235 microtome, Germany). Ten serial sections per animal were stained with haematoxylin and eosin (H&E). Quantitative analysis of the staining was carried out using an Olympus BX-50 microscope, fitted with a motorised specimen stage and microcator (Olympus, Tokyo, Japan). All analysis was performed with the Computer Assisted Stereology Toolbox (CAST) version 2.0 program (Olympus, Ballerup, Denmark), with the observer blind to the treatment groups. Uterine artery area was determined using a point grid, which was superimposed on the sections and viewed using a × 20 objective. Points falling on the wall or lumen of the vessel were counted and the areas were calculated as:

$$A(obj) = a(p) \times \Sigma p$$

Where A(obj) is the estimated area, a(p) is the area associated with each point and Σp is the sum of points falling on the relevant area, averaged over the sections.

The lumen diameter was calculated as the maximum perpendicular distance across the lumen and the external diameter of the vessels was measured at the same position as the lumen diameter using the CAST program. These thicknesses were estimated using a line grid that was superimposed on sections to establish random start points using the method of orthogonal intercepts viewed with a × 20 objective (Camm *et al.*, 2010). The density of smooth muscle cells (SMCs) nuclei (an indicator of hyperplasia) in the wall was investigated in 5 H&E-stained sections using a ×100 objective, the number of SMCs nuclei within a counting frame (617 μm^2) was determined in 10 fields per section. To establish the area of the uterine artery wall occupied by SMCs nuclei, an indicator of hypertrophy, the ratio of the relative wall area/SMCs nuclei number was determined (Camm *et al.*, 2010).

Statistical Analyses

Appropriate power calculations derived from previous data sets were performed to determine the minimum sample size required to achieve statistical significance (Camm *et al.*, 2010; Herrera *et al.*, 2011; Giussani *et al.*, 2012; Richter *et al.*, 2012; Kane *et al.*, 2013; Allison *et al.*, 2016; Niu *et al.*, 2018; Nuzzo *et al.*, 2018; Camm *et al.*, 2021; Spiroski *et al.*, 2021; Hansell *et al.*, 2022). The experiments were completed within one experimental season, and scientists measuring outcomes were blinded to treatments. Data are expressed as means ± SEM and were analysed either using the Generalised Linear Model or Two-way analysis of variation (ANOVA) with the Tukey's *post hoc* test when appropriate (IBM SPSS Statistics 20.0, Chicago, IL, USA). For all comparisons, statistical significance was accepted when $P < 0.05$.

RESULTS

Maternal and Offspring Biometry and MitoQ Tissue Uptake

Hypoxic pregnancy with or without maternal treatment with MitoQ increased placental weight without an effect on overall maternal food intake, maternal weight, fetal weight, or litter size (Table 1). Maternal treatment with MitoQ in normoxic or hypoxic pregnancy reduced maternal water intake (Table 1). Maternal treatment with MitoQ was taken up in all tissues measured in both normoxic and hypoxic pregnancy (Figure 2). The uptake of MitoQ in tissues was not significantly different between normoxic compared to hypoxic pregnancy (Figure 2).

Uterine Artery Vasoconstrictor Function

Relative to normoxic pregnancy, uterine artery constrictor responses to NE (Fig. 3A and 3B), Ang II (Fig. 3C and 3D) and PDBu (Fig. 3E and 3F) were all significantly enhanced in hypoxic pregnancy. Maternal treatment with MitoQ prevented the effect of hypoxic pregnancy on all constrictor agents (Fig. 3A-F). In contrast, maternal treatment with MitoQ in normoxic pregnancy did not have any effect (Fig. 3A-F).

Uterine Artery Vasodilator Function

Relative to normoxic pregnancy, uterine artery relaxant responses to SNP (Fig. 4A and 4B), ACh (Fig. 4C and 4D) and NS1619 (Fig. 4E and 4F) were all significantly enhanced in hypoxic pregnancy. Deeper analysis of NO-dependent and NO-independent mechanisms contributing to ACh-induced vasorelaxation revealed that hypoxic pregnancy enhanced endothelial relaxation by increasing NO-dependent components (black histogram; Fig. 4D). Maternal treatment with MitoQ prevented the effect of hypoxic pregnancy on all dilator agents (Fig. 4A-F). Maternal treatment with MitoQ in normoxic pregnancy had no effect on SNP or NS1619 but significantly increased the ACh-induced vasorelaxation by increasing NO-dependent mechanisms (black histogram; Fig. 4D).

Uterine Artery Morphology

Relative to normoxic pregnancy, uterine arteries isolated from hypoxic pregnancy had greater

wall thickness and an increased ratio of the wall area to lumen area (Fig. 5A and 5B). When expressed as a percentage of the total vessel area, uterine arteries from hypoxic pregnancy had significantly reduced lumen area (white histogram) and significantly enhanced wall area (black histogram; Fig. 5C). These effects of hypoxic pregnancy occurred without an effect on uterine smooth muscle cell nuclear density (N=12.2±0.9 %, H=10.7±1.1 %, HQ=12.8±0.3 %, NQ=10.4±0.4 %; P=0.6156). Therefore, the relative uterine wall area divided by the estimated cell number, an index of cellular hypertrophy, was significantly increased in hypoxic pregnancy (N=0.45±0.05, H=0.65±0.07, HQ=0.47±0.06, NQ=0.44±0.05; N *vs*. H, P=0.0482). Maternal treatment with MitoQ prevented all effects in hypoxic pregnancy on uterine vascular remodelling and it had no effect in normoxic pregnancy (Fig. 5A-5D). There was a tendency for MitoQ treatment to increase uterine artery diameter in normoxic (0.311±0.028 *vs.* 0.358±0.021 mm, P=0.6799) and hypoxic (0.289±0.036 *vs.* 0.366±0.038 mm, P=0.3482) pregnancy. However, this effect did not reach statistical significance (Two-way ANOVA).

DISCUSSION

The data in the present study show that hypoxic pregnancy in rats increased uterine artery constrictor reactivity to norepinephrine, angiotensin II, and the protein kinase C activator PDBu, near term. In turn, chronic hypoxia during pregnancy also enhanced uterine artery dilator reactivity to nitroprusside and acetylcholine (ACh) near term. Deeper vascular experiments and analysis revealed that the enhanced endothelium-dependent reactivity to acetylcholine is mediated via increased NO-dependent mechanisms and activation of the large conductance Ca^{2+}-activated K^{+}-channel (BKCa). Uterine arteries isolated from hypoxic pregnancy near term also showed greater wall thickness with reduced lumen area, and an increase in the uterine wall area divided by the estimated cell number, findings consistent with vessel concentric hypertrophy. Maternal *in vivo* treatment with MitoQ during hypoxic pregnancy normalised uterine artery constrictor and dilator reactivity and prevented the uterine artery vascular remodelling. Maternal *in vivo* MitoQ treatment in normoxic pregnancy did not have an effect on uterine artery constriction or remodelling, however it also enhanced dilator reactivity to ACh via increased NO-dependent mechanisms near term. Maternal treatment with MitoQ in normoxic or hypoxic pregnancy tended to increase uterine artery diameter, however this effect did not reach statistical significance. Combined, these data support that hypoxic pregnancy sensitises uterine vascular bed vasoconstrictor responses mediated via protein kinase C signalling and triggers up-regulation of compensatory dilator mechanisms. Maternal *in vivo* MitoQ treatment prevents the hypoxia-induced increase in uterine artery constrictor reactivity and remodelling, negating the need for enhanced uterine artery dilator compensation. Therefore, the data support the hypothesis that maternal *in vivo* oral treatment with MitoQ protects against uterine artery vasoconstrictor hyper-reactivity and vascular remodelling in pregnancy complicated by gestational hypoxia.

Protein Kinase C (PKC) is a ubiquitous enzyme found in almost all cell types including the endothelium and smooth muscle of blood vessels. PKC is activated by alpha-adrenergic agonists and by angiotensin II (Ringvold & Khalil, 2017). Studies of uterine arteries isolated from pregnant sheep exposed to high altitude during pregnancy reported that the chronic

hypoxia-induced depression in α_1-adrenergic receptor constrictor reactivity is mediated, at least in part, by a fall in α_1-adrenergic receptor density, agonist binding affinity and coupling efficiency to InsP3 synthesis in the uterine artery (Hu *et al.*, 1996; Hu *et al.*, 1999). Further studies in this ovine model report that chronic hypoxia enhances the endothelium-dependent relaxation to the calcium ionophore A23187 in precontracted uterine vessels (Xiao *et al.*, 2001). These changes are associated with increased endothelial nitric oxide synthase (eNOS) protein expression in the uterine artery of highland sheep (Xiao *et al.*, 2001). Studies of uterine arteries isolated from pregnant guinea pigs exposed to high altitude during pregnancy report that chronic hypoxia does not diminish the pregnancy-associated reduction in contractile sensitivity to phenylephrine but enhances basal nitric oxide activity in the pregnant mesenteric artery (White *et al.*, 1998; White *et al.*, 2000). Data in the present rat study suggest that hypoxic pregnancy enhances constrictor reactivity to the PKC activator PBDu, norepinephrine and angiotensin II, and triggers an increase in endothelium-dependent reactivity mediated in part via increased NO-dependent mechanisms and activation of the large conductance Ca^{2+}-activated K^+-channel (BKCa) in the uterine artery. Further, maternal *in vivo* MitoQ treatment prevents the enhanced constrictor reactivity and normalises dilator responses in uterine arteries from hypoxic pregnant rats. Therefore, while the effects of hypoxic pregnancy on constrictor reactivity in the uterine may vary, past and present data support that hypoxic pregnancy in preclinical mammalian models trigger compensatory up-regulation of dilator mechanisms in the uterine artery which involve enhanced NO signalling and activation of large conductance Ca^{2+}-activated K^+-channels (BKCa). In addition, past (Hu *et al.*, 2023) and present data show that MitoQ treatment may not only protect against enhanced myogenic tone, but it may also normalise constrictor and dilator reactivity to agonists in the uterine artery during hypoxic pregnancy. Further, maternal MitoQ treatment in normoxic pregnancy enhances NO-dependent uterine artery dilator reactivity. This suggests that in healthy pregnancy, mitochondria-derived ROS generation limits NO bioavailability and dilator capacity, and that this limitation can be removed with mitochondria-targeted antioxidants.

Uterine artery diameter is a major determinant of uterine blood flow, and sustained changes in uterine artery blood flow are important in vascular growth and remodeling (Mulvany *et al.*, 1996; Pourageaud & Mey, 1997). For instance, outward hypertrophic growth occurs with increased flow, whereas inward hypertrophic growth and a decrease in lumenal diameter result from downstream increases in peripheral vascular resistance, hypertension, and an increase in cardiac afterload (Mulvany *et al.*, 1996; Pourageaud & Mey, 1997). Zamudio and colleagues (Zamudio *et al.*, 1995a) reported lower near-term uterine artery blood flow in pregnant women residing at a high altitude compared with lowland pregnant women, owing primarily to a decrease in vessel diameter resulting from structural remodeling of the uterine artery. Residence at high altitude also increases the incidence of fetal growth restriction and pre-eclampsia (Jensen & Moore, 1997; Palmer *et al.*, 1999; Giussani *et al.*, 2001; Soria *et al.*, 2013), conditions associated with reduced uteroplacental blood flow. Preeclamptic women demonstrate an absence or reversal of the normal diminution in arterial blood pressure and greater vasoconstrictor response to angiotensin II, suggesting that preeclampsia interferes with the normal vascular adjustment to pregnancy (Gant *et al.*, 1977). Similarly, Hu and colleagues (Hu *et al.*, 2017) report that sheep undergoing pregnancy at high altitude display greater basal arterial blood pressure and uterine vascular resistance and fail to show the expected fall in arterial blood pressure with advancing gestation when compared to sea level pregnant sheep. In addition, we previously reported that pregnant sheep exposed to isobaric chronic hypoxia for the last third of pregnancy failed to show the expected fall in uterine vascular resistance and maternal arterial blood pressure with advancing gestation (Tong *et al.*, 2022). However, an increase in oxidative stress and activation of the unfolded protein response in the placenta was evident. The present study shows that *in vivo* maternal MitoQ treatment in hypoxic pregnancy in rats prevented concentric hypertrophic growth in the uterine artery, consistent with the effects of MitoQ normalising uterine artery constrictor hyper-reactivity and the oxidative stress that mediates uterine artery vascular dysfunction and remodelling.

In rat pregnancy, late-onset hypoxia (10-11% O_2) from day 15 of gestation (term is *ca*. 21 days) induces marked fetal growth restriction (Camm *et al.*, 2010; Hansell *et al.*, 2022), whereas

359 early-onset hypoxia (13-14% O_2) from day 6 of gestation does not (Camm *et al.*, 2010;
360 Giussani *et al.*, 2012). This is because early-onset hypoxia triggers placental adaptations to
361 protect against the adverse effects of chronic hypoxia on fetal growth (Giussani *et al.*, 2012;
362 Richter *et al.*, 2012; Nuzzo *et al.*, 2018). Late-onset hypoxia does not have the same effect, as
363 the challenge occurs following placentation and maximal placental growth (Adler *et al.*, 2010;
364 Giussani *et al.*, 2012; Giussani & Davidge, 2013). Data in the present study show that early
365 onset hypoxia in rats leads to uterine vascular dysfunction and remodelling independent of
366 effects on fetal growth. Therefore, the data support that uterine vascular dysfunction and
367 remodelling are direct effect of gestational hypoxia, and that these changes can occur in the
368 absence of fetal growth restriction. Past and present data also highlight that despite overt
369 uterine concentric hypertrophic growth and a fall in uterine artery lumenal diameter, uterine
370 artery compensatory dilator responses and placental adaptations can act in concert to
371 ameliorate the negative effects of pregnancy complicated by chronic hypoxia on fetal growth
372 (Giussani *et al.*, 2012; Richter *et al.*, 2012; Nuzzo *et al.*, 2018)

373 In the present study, maternal water intake was slightly reduced by maternal treatment with
374 MitoQ in both normoxic and hypoxic pregnancy. This is a known effect of MitoQ in rodent
375 studies (Nuzzo *et al.*, 2018; Spiroski *et al.*, 2021), likely due to the palatability of oral MitoQ
376 provided in drinking water. Therefore, the dose regimen in the present study was adjusted
377 for efficacy accounting for this effect. However, MitoQ administration in human clinical trials
378 is via tablet (Gane *et al.*, 2010; Snow *et al.*, 2010), resolving any potential issues with taste
379 adversity.

380 **Perspectives**

381 Many adverse conditions during pregnancy including pregnancy at high altitude, preeclampsia,
382 chorioamnionitis, gestational diabetes and maternal obesity can trigger utero-placental hypoxia
383 (Giussani & Davidge, 2013; Tong & Giussani, 2019; Giussani, 2021). In turn, utero-placental
384 hypoxia has been associated with oxidative stress in the maternal, placental, and fetal tissues,
385 triggering adverse consequences on the physiology of the mother and offspring that can

increase their cardiovascular risk in later life (Giussani & Davidge, 2013; Tong & Giussani, 2019; Botting *et al.*, 2020; Giussani, 2021). Here we show that the mitochondria-targeted antioxidant MitoQ can be successfully administered *in vivo* orally in pregnancy and that it can also protect the maternal uterine circulation against vascular dysfunction and remodelling in hypoxic pregnancy. Therefore, maternal MitoQ treatment in adverse pregnancy may not only have beneficial effects on the offspring, but its protective effects transcend to the maternal uterine circulation in hypoxic pregnancy. Whether maternal oral *in vivo* treatment with MitoQ may prove similarly protective against uterine vascular dysfunction and remodelling in other types of complicated pregnancy, including advanced maternal age (Pirhonen *et al.*, 2005; Care *et al.*, 2015), maternal hypertension of pregnancy (Myatt & Webster, 2009; Brennan *et al.*, 2014), maternal obesity, gestational diabetes (Stanley *et al.*, 2009; Goulopoulou *et al.*, 2014) and pregnancy at high altitude (Zamudio *et al.*, 1995b) awaits investigation.

Acknowledgements

We are grateful to the staff of the University of Cambridge Biological Services for helping with the maintenance of the animals. D.A.G. is a Professorial Fellow at Gonville & Caius College, a Fellow of the Lister Institute for Preventive Medicine, and a Royal Society Wolfson Research Merit Award Holder.

Sources of Funding

This work was supported by the British Heart Foundation (RG/17/8/32924 and PG/14/5/30547; D.A.G.), the Medical Research Council UK (MC_UU_00028/4; M.P.M.) and by a Wellcome Trust Investigator award (220257/Z/20/Z; M.P.M.).

Disclosures

The authors declare no competing interests.

Author contributions

Conceptualization: D.A.G., Y.N., Z.W., J.M., M.P.M; Methodology: Z.W., E.J.C., A.M.N., A.S., K.L.S., T.J.A., A.R., T.T., A.L., J.M., M.P.M., Y.N., D.A.G; Data and Statistical Analysis: Z.W., E.J.C., A.M.N., A.S., K.L.S., T.J.A., A.R., T.T., A.L., J.M., M.P.M., Y.N., D.A.G; Writing – Original Draft: Z.W., D.A.G., Y.N. , M.P.M; Writing – Review & Editing: Z.W., E.J.C., A.M.N., A.S., K.L.S., T.J.A., A.R., T.T., A.L., J.M., M.P.M., Y.N., D.A.G; Visualization: Z.W., E.J.C., A.M.N., A.S., K.L.S., T.J.A., A.R., T.T., A.L., J.M., M.P.M., Y.N., D.A.G; Supervision: D.A.G., Y.N., E.J.C., J.M., M.P.M; Project Administration: D.A.G., Y.N., E.J.C., J.M., M.P.M; Funding Acquisition: D.A.G.

Data availability statement

All data are presented within the paper. Further information and requests for resources and reagents should be directed to the Lead Contact: Professor Dino A. Giussani (dag26@cam.ac.uk).

Materials availability

This study did not generate new unique reagents.

Data and code availability

This study did not generate/analyse any datasets or code.

REFERENCES

Adler A, Camm EJ, Hansell JA, Richter HG & Giussani DA. (2010). Investigation of the Use of Antioxidants to Diminish the Adverse Effects of Postnatal Glucocorticoid Treatment on Mortality and Cardiac Development. *Neonatology* **98,** 73-83.

Allison BJ, Kaandorp JJ, Kane AD, Camm EJ, Lusby C, Cross CM, Nevin-Dolan R, Thakor AS, Derks JB, Tarry-Adkins JL, Ozanne SE & Giussani DA. (2016). Divergence of mechanistic pathways mediating cardiovascular aging and developmental programming of cardiovascular disease. *The FASEB Journal* **30,** 1968-1975.

Botting KJ, Skeffington KL, Niu Y, Allison BJ, Brain KL, Itani N, Beck C, Logan A, Murray AJ, Murphy MP & Giussani DA. (2020). Translatable mitochondria-targeted protection against programmed cardiovascular dysfunction. *Science Advances* **6,** eabb1929.

Brennan LJ, Morton JS & Davidge ST. (2014). Vascular Dysfunction in Preeclampsia. *Microcirculation* **21,** 4-14.

Camm EJ, Cross CM, Kane AD, Tarry-Adkins JL, Ozanne SE & Giussani DA. (2021). Maternal antioxidant treatment protects adult offspring against memory loss and hippocampal atrophy in a rodent model of developmental hypoxia. *The FASEB Journal* **35,** e21477.

Camm EJ, Hansell JA, Kane AD, Herrera EA, Lewis C, Wong S, Morrell NW & Giussani DA. (2010). Partial contributions of developmental hypoxia and undernutrition to prenatal alterations in somatic growth and cardiovascular structure and function. *Am J Obstet Gynecol* **203,** 495.e424-495.e434.

Care AS, Bourque SL, Morton JS, Hjartarson EP & Davidge ST. (2015). Effect of Advanced Maternal Age on Pregnancy Outcomes and Vascular Function in the Rat. *Hypertension* **65,** 1324-1330.

Chen K & Keaney J. (2004). Reactive oxygen species-mediated signal transduction in the endothelium. *Endothelium : journal of endothelial cell research* **11,** 109-121.

Cochemé HM, Kelso GF, James AM, Ross MF, Trnka J, Mahendiran T, Asin-Cayuela J, Blaikie FH, Manas A-RB, Porteous CM, Adlam VJ, Smith RAJ & Murphy MP. (2007). Mitochondrial targeting of quinones: Therapeutic implications. *Mitochondrion* **7,** S94-S102.

Duvekot JJ & Peeters LLH. (1994). Maternal Cardiovascular Hemodynamic Adaptation to Pregnancy. *Obstetrical & Gynecological Survey* **49,** S1.

Gandley RE, Conrad KP & McLaughlin MK. (2001). Endothelin and nitric oxide mediate

reduced myogenic reactivity of small renal arteries from pregnant rats. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **280,** R1-R7.

Gane EJ, Weilert F, Orr DW, Keogh GF, Gibson M, Lockhart MM, Frampton CM, Taylor KM, Smith RAJ & Murphy MP. (2010). The mitochondria-targeted anti-oxidant mitoquinone decreases liver damage in a phase II study of hepatitis C patients. *Liver International* **30,** 1019-1026.

Ganguly E, Kirschenman R, Spaans F, Holody CD, Phillips TEJ, Case CP, Cooke C-LM, Murphy MP, Lemieux H & Davidge ST. (2021). Nanoparticle-encapsulated antioxidant improves placental mitochondrial function in a sexually dimorphic manner in a rat model of prenatal hypoxia. *The FASEB Journal* **35,** e21338.

Gant NF, Jimenez JM, Whalley PJ, Chand S & MacDonald PC. (1977). A prospective study of angiotensin II pressor responsiveness in pregnancies complicated by chronic essential hypertension. *American Journal of Obstetrics & Gynecology* **127,** 369-375.

Giussani DA. (2021). Breath of Life: Heart Disease Link to Developmental Hypoxia. *Circulation* **144,** 1429-1443.

Giussani DA, Camm EJ, Niu Y, Richter HG, Blanco CE, Gottschalk R, Blake EZ, Horder KA, Thakor AS, Hansell JA, Kane AD, Wooding FBP, Cross CM & Herrera EA. (2012). Developmental Programming of Cardiovascular Dysfunction by Prenatal Hypoxia and Oxidative Stress. *PLoS ONE* **7,** e31017.

Giussani DA & Davidge ST. (2013). Developmental programming of cardiovascular disease by prenatal hypoxia. *Journal of Developmental Origins of Health and Disease* **4,** 328-337.

Giussani DA, Phillips PS, Anstee S & Barker DJP. (2001). Effects of Altitude versus Economic Status on Birth Weight and Body Shape at Birth. *Pediatr Res* **49,** 490-494.

Gluckman PD, Hanson MA, Cooper C & Thornburg KL. (2008). Effect of In Utero and Early-Life Conditions on Adult Health and Disease. *N Engl J Med* **359,** 61-73.

Goulopoulou S, Hannan JL, Matsumoto T, Ergul A & Webb RC. (2014). Augmented dilation to nitric oxide in uterine arteries from rats with type 2 diabetes: implications for vascular adaptations to pregnancy. *American Journal of Physiology-Heart and Circulatory Physiology* **306,** H610-H618.

Graham D, Huynh NN, Hamilton CA, Beattie E, Smith RAJ, Cochemé HM, Murphy MP & Dominiczak AF. (2009). Mitochondria-Targeted Antioxidant MitoQ₁₀ Improves Endothelial Function and Attenuates Cardiac Hypertrophy. *Hypertension* **54,**

322-328.

Hansell JA, Richter HG, Camm EJ, Herrera EA, Blanco CE, Villamor E, Patey OV, Lock MC, Trafford AW, Galli GLJ & Giussani DA. (2022). Maternal melatonin: Effective intervention against developmental programming of cardiovascular dysfunction in adult offspring of complicated pregnancy. *Journal of Pineal Research* **72,** e12766.

Herrera EA, Camm EJ, Cross CM, Mullender JL, Wooding FBP & Giussani DA. (2011). Morphological and Functional Alterations in the Aorta of the Chronically Hypoxic Fetal Rat. *Journal of Vascular Research* **49,** 50-58.

Hu X-Q, Dasgupta C, Xiao D, Huang X, Yang S & Zhang L. (2017). MicroRNA-210 Targets Ten-Eleven Translocation Methylcytosine Dioxygenase 1 and Suppresses Pregnancy-Mediated Adaptation of Large Conductance Ca²⁺-Activated K⁺ Channel Expression and Function in Ovine Uterine Arteries. *Hypertension* **70,** 601-612.

Hu X-Q, Song R, Dasgupta C, Blood AB & Zhang L. (2023). TET2 confers a mechanistic link of microRNA-210 and mtROS in hypoxia-suppressed spontaneous transient outward currents in uterine arteries of pregnant sheep. *The Journal of Physiology* **601,** 1501-1514.

Hu X-Q & Zhang L. (2021). Hypoxia and Mitochondrial Dysfunction in Pregnancy Complications. *Antioxidants* **10,** 405.

Hu XQ, Longo LD, Gilbert RD & Zhang L. (1996). Effects of long-term high-altitude hypoxemia on alpha 1-adrenergic receptors in the ovine uterine artery. *American Journal of Physiology-Heart and Circulatory Physiology* **270,** H1001-H1007.

Hu XQ, Yang S, Pearce WJ, Longo LD & Zhang L. (1999). Effect of Chronic Hypoxia on Alpha-1 Adrenoceptor-Mediated Inositol 1,4,5-Trisphosphate Signaling in Ovine Uterine Artery. *Journal of Pharmacology and Experimental Therapeutics* **288,** 977.

Hu XQ & Zhang L. (1997). Chronic hypoxia suppresses pharmacomechanical coupling of the uterine artery in near-term pregnant sheep. *The Journal of Physiology* **499,** 551-559.

Jensen GM & Moore LG. (1997). The effect of high altitude and other risk factors on birthweight: independent or interactive effects? *American Journal of Public Health* **87,** 1003-1007.

Kane AD, Herrera EA, Camm EJ & Giussani DA. (2013). Vitamin C Prevents Intrauterine Programming of in vivo Cardiovascular Dysfunction in the Rat. *Circulation Journal* **77,** 2604-2611.

Keyes LE, Armaza FJ, Niermeyer S, Vargas E, Young DA & Moore LG. (2003). Intrauterine Growth Restriction, Preeclampsia, and Intrauterine Mortality at High Altitude in Bolivia. *Pediatr Res* **54,** 20-25.

Kilkenny C, Browne WJ, Cuthill IC, Emerson M & Altman DG. (2010). Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. *PLOS Biology* **8,** e1000412.

Kublickiene KR, Cockell AP, Nisell H & Poston L. (1997). Role of nitric oxide in the regulation of vascular tone in pressurized and perfused resistance myometrial arteries from term pregnant women. *Am J Obstet Gynecol* **177,** 1263-1269.

Longo LD. (1983). Maternal blood volume and cardiac output during pregnancy: a hypothesis of endocrinologic control. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **245,** R720-R729.

Mandala M & Osol G. (2012). Physiological Remodelling of the Maternal Uterine Circulation during Pregnancy. *Basic & Clinical Pharmacology & Toxicology* **110,** 12-18.

Meyer MC, Brayden JE & McLaughlin MK. (1993). Characteristics of vascular smooth muscle in the maternal resistance circulation during pregnancy in the rat. *Am J Obstet Gynecol* **169,** 1510-1516.

Meyer MC, Osol G & McLaughlin M. (1997). Flow Decreases Myogenic Reactivity of Mesenteric Arteries From Pregnant Rats. *Journal of the Society for Gynecologic Investigation* **4,** 293-297.

Moore LG, Hershey DW, Jahnigen D & Bowes W. (1982). The incidence of pregnancy-induced hypertension is increased among Colorado residents at high altitude. *Am J Obstet Gynecol* **144,** 423-429.

Morton JS, Care AS & Davidge ST. (2017). Mechanisms of Uterine Artery Dysfunction in Pregnancy Complications. *Journal of Cardiovascular Pharmacology* **69,** 343-359.

Mulvany MJ, Baumbach GL, Aalkjaer C, Heagerty AM, Korsgaard N, Schiffrin EL & Heistad DD. (1996). Vascular remodeling. *Hypertension* **28,** 505-506.

Myatt L & Webster RP. (2009). Vascular biology of preeclampsia. *Journal of Thrombosis and Haemostasis* **7,** 375-384.

Ni Y, Meyer M & Osol G. (1997). Gestation increases nitric oxide–mediated vasodilation in rat uterine arteries. *Am J Obstet Gynecol* **176,** 856-864.

Niu Y, Kane AD, Lusby CM, Allison BJ, Chua YY, Kaandorp JJ, Nevin-Dolan R, Ashmore TJ, Blackmore HL, Derks JB, Ozanne SE & Giussani DA. (2018). Maternal Allopurinol Prevents Cardiac Dysfunction in Adult Male Offspring Programmed by Chronic Hypoxia During Pregnancy. *Hypertension* **72,** 971-978.

Nuzzo AM, Camm EJ, Sferruzzi-Perri AN, Ashmore TJ, Yung H-w, Cindrova-Davies T, Spiroski A-M, Sutherland MR, Logan A, Austin-Williams S, Burton GJ, Rolfo A, Todros T, Murphy MP & Giussani DA. (2018). Placental Adaptation to Early-Onset Hypoxic Pregnancy and Mitochondria-Targeted Antioxidant Therapy in a Rodent Model. *The American Journal of Pathology* **188,** 2704-2716.

Osol G & Cipolla M. (1993). Interaction of myogenic and adrenergic mechanisms in isolated, pressurized uterine radial arteries from late-pregnant and nonpregnant rats. *Am J Obstet Gynecol* **168,** 697-705.

Palmer SK, Moore LG, Young DA, Cregger B, Berman JC & Zamudio S. (1999). Altered blood pressure course during normal pregnancy and increased preeclampsia at high altitude (3100 meters) in Colorado. *Am J Obstet Gynecol* **180,** 1161-1168.

Pirhonen J, Bergersen TK, Abdlenoor M, Dubiel M & Gudmundsson S. (2005). Effect of maternal age on uterine flow impedance. *Journal of Clinical Ultrasound* **33,** 14-17.

Pourageaud F & Mey JGRD. (1997). Structural properties of rat mesenteric small arteries after 4-wk exposure to elevated or reduced blood flow. *American Journal of Physiology-Heart and Circulatory Physiology* **273,** H1699-H1706.

Richter HG, Camm EJ, Modi BN, Naeem F, Cross CM, Cindrova-Davies T, Spasic-Boskovic O, Dunster C, Mudway IS, Kelly FJ, Burton GJ, Poston L & Giussani DA. (2012). Ascorbate prevents placental oxidative stress and enhances birth weight in hypoxic pregnancy in rats. *The Journal of Physiology* **590,** 1377-1387.

Ringvold HC & Khalil RA. (2017). Chapter Six - Protein Kinase C as Regulator of Vascular Smooth Muscle Function and Potential Target in Vascular Disorders. In *Advances in Pharmacology*, ed. Khalil RA, pp. 203-301. Academic Press.

Rodriguez-Cuenca S, Cochemé HM, Logan A, Abakumova I, Prime TA, Rose C, Vidal-Puig A, Smith AC, Rubinsztein DC, Fearnley IM, Jones BA, Pope S, Heales SJR, Lam BYH, Neogi SG, McFarlane I, James AM, Smith RAJ & Murphy MP. (2010). Consequences of long-term oral administration of the mitochondria-targeted antioxidant MitoQ to wild-type mice. *Free Radical Biology and Medicine* **48,** 161-172.

Rosenfeld CR. (1977). Distribution of cardiac output in ovine pregnancy. *American Journal of Physiology-Heart and Circulatory Physiology* **232,** H231-H235.

637 Sanghavi M & Rutherford JD. (2014). Cardiovascular Physiology of Pregnancy. *Circulation*
638 **130,** 1003-1008.
639
640 Skeffington KL, Higgins JS, Mahmoud AD, Evans AM, Sferruzzi-Perri AN, Fowden AL, Yung
641 HW, Burton GJ, Giussani DA & Moore LG. (2016). Hypoxia, AMPK activation and
642 uterine artery vasoreactivity. *The Journal of Physiology* **594,** 1357-1369.
643
644 Smith RAJ & Murphy MP. (2010). Animal and human studies with the mitochondria-targeted
645 antioxidant MitoQ. *Annals of the New York Academy of Sciences* **1201,** 96-103.
646
647 Snow BJ, Rolfe FL, Lockhart MM, Frampton CM, O'Sullivan JD, Fung V, Smith RAJ, Murphy
648 MP, Taylor KM & Group ObotPS. (2010). A double-blind, placebo-controlled study to
649 assess the mitochondria-targeted antioxidant MitoQ as a disease-modifying therapy in
650 Parkinson's disease. *Movement Disorders* **25,** 1670-1674.
651
652 Soria R, Julian CG, Vargas E, Moore LG & Giussani DA. (2013). Graduated effects of high-
653 altitude hypoxia and highland ancestry on birth size. *Pediatr Res* **74,** 633-638.
654
655 Spiroski A-M, Niu Y, Nicholas LM, Austin-Williams S, Camm EJ, Sutherland MR, Ashmore
656 TJ, Skeffington KL, Logan A, Ozanne SE, Murphy MP & Giussani DA. (2021).
657 Mitochondria antioxidant protection against cardiovascular dysfunction programmed
658 by early-onset gestational hypoxia. *The FASEB Journal* **35,** e21446.
659
660 Stanley JL, Ashton N, Taggart MJ, Davidge ST & Baker PN. (2009). Uterine artery function
661 in a mouse model of pregnancy complicated by diabetes. *Vascular Pharmacology* **50,**
662 8-13.
663
664 Thakor AS, Richter HG, Kane AD, Dunster C, Kelly FJ, Poston L & Giussani DA. (2010).
665 Redox modulation of the fetal cardiovascular defence to hypoxaemia. *The Journal of*
666 *Physiology* **588,** 4235-4247.
667
668 Thompson LP & Al-Hasan Y. (2012). Impact of Oxidative Stress in Fetal Programming.
669 *Journal of Pregnancy* **2012,** 582748.
670
671 Thornburg KL, Jacobson S-L, Giraud GD & Morton MJ. (2000). Hemodynamic changes in
672 pregnancy. *Seminars in Perinatology* **24,** 11-14.
673
674 Tong W, Allison BJ, Brain KL, Patey OV, Niu Y, Botting KJ, Ford SG, Garrud TA, Wooding
675 PFB, Shaw CJ, Lyu Q, Zhang L, Ma J, Cindrova-Davies T, Yung HW, Burton GJ &
676 Giussani DA. (2022). Chronic Hypoxia in Ovine Pregnancy Recapitulates
677 Physiological and Molecular Markers of Preeclampsia in the Mother, Placenta, and
678 Offspring. *Hypertension* **79,** 1525-1535.
679
680 Tong W & Giussani DA. (2019). Preeclampsia link to gestational hypoxia. *Journal of*

Developmental Origins of Health and Disease **10,** 322-333.

Veerareddy S, Cooke C-LM, Baker PN & Davidge ST. (2002). Vascular adaptations to pregnancy in mice: effects on myogenic tone. *American Journal of Physiology-Heart and Circulatory Physiology* **283,** H2226-H2233.

Weiner C, Liu KZ, Thompson L, Herrig J & Chestnut D. (1991). Effect of pregnancy on endothelium and smooth muscle: their role in reduced adrenergic sensitivity. *American Journal of Physiology-Heart and Circulatory Physiology* **261,** H1275-H1283.

Weiner CP, Thompson LP, Liu K-Z & Herrig JE. (1992). Endothelium-derived relaxing factor and indomethacin-sensitive contracting factor alter arterial contractile responses to thromboxane during pregnancy. *Am J Obstet Gynecol* **166,** 1171-1181.

White MM, McCullough RE, Dyckes R, Robertson AD & Moore LG. (1998). Effects of pregnancy and chronic hypoxia on contractile responsiveness to α1-adrenergic stimulation. *J Appl Physiol* **85,** 2322-2329.

White MM, McCullough RE, Dyckes R, Robertson AD & Moore LG. (2000). Chronic hypoxia, pregnancy, and endothelium-mediated relaxation in guinea pig uterine and thoracic arteries. *American Journal of Physiology-Heart and Circulatory Physiology* **278,** H2069-H2075.

White MM & Zhang L. (2003). Effects of Chronic Hypoxia on Maternal Vasodilation and Vascular Reactivity in Guinea Pig and Ovine Pregnancy. *High Altitude Medicine & Biology* **4,** 157-169.

Xiao D, Bird IM, Magness RR, Longo LD & Zhang L. (2001). Upregulation of eNOS in pregnant ovine uterine arteries by chronic hypoxia. *American Journal of Physiology-Heart and Circulatory Physiology* **280,** H812-H820.

Xiao D, Buchholz JN & Zhang L. (2006). Pregnancy attenuates uterine artery pressure-dependent vascular tone: role of PKC/ERK pathway. *American Journal of Physiology-Heart and Circulatory Physiology* **290,** H2337-H2343.

Zamudio S, Palmer SK, Dahms TE, Berman JC, Young DA & Moore LG. (1995a). Alterations in uteroplacental blood flow precede hypertension in preeclampsia at high altitude. *J Appl Physiol* **79,** 15-22.

Zamudio S, Palmer SK, Droma T, Stamm E, Coffin C & Moore LG. (1995b). Effect of altitude on uterine artery blood flow during normal pregnancy. *J Appl Physiol* **79,** 7-14.

Table 1. Maternal pregnancy and litter characteristics

	N	H	HQ	NQ
Maternal weight (g)	390.71±6.98 (n=10)	385.03±9.11 (n=11)	377.51±7.56 (n=12)	390.56±9.45 (n=12)
Fetal weight (all) (g)	3.45±0.34 (n=154)	3.39±0.28 (n=172)	3.49±0.29 (n=157)	3.38±0.33 (n=193)
Placental weight (all) (g)	0.53±0.01 (n=120)	0.62±0.01* (n=139)	0.61±0.01* (n=129)	0.52±0.01 (n=155)
Litter size (n)	15.09±0.72 (n=11 dams)	15.73±0.78 (n=11 dams)	14.36±0.73 (n=11 dams)	16.25±0.87 (n=12 dams)
Water intake ($ml.kg.d^{-1}$)	176±8 (n=10 dams)	173±9 (n=10 dams)	125±10 (n=6 dams)	146±9 (n=8 dams)
Food intake ($g.kg.d^{-1}$)	79±2 (n=10 dams)	70±3 (n=10 dams)	73±3 (n=6 dams)	75±3 (n=8 dams)

Maternal, fetal and placental weights, litter sizes, and maternal water and food intake from normoxic (N), hypoxic (H), hypoxic+MitoQ (HQ) and normoxic+MitoQ (NQ) rodent pregnancy. Values are mean±S.E.M. *P<0.05 *vs*. N and NQ (Generalised linear model).

LEGENDS

Abstract Figure legend. Moderate hypoxia (13% O_2) during rat pregnancy programs uterine artery remodeling and dysfunction via mitochondria derived oxidative stress. The mitochondria-targeted antioxidant MitoQ treatment prevents the adverse changes in uterine artery structure and function in high-risk pregnancy.

Figure 1. Experimental protocols. Panel A: Pregnant dams were singly housed and randomly assigned to either Normoxia (N, 21% O2), Hypoxia (H, 13% O2), Hypoxia+MitoQ (HQ, 13% O2) or Normoxia+MitoQ (NQ, 21% O2) treatment from day 6 until day 20 of pregnancy. N=11-12 per group. MitoQ was administered in the drinking water at a concentration of 500 μM. On day 20, all dams were returned to normoxic conditions and provided normal drinking water. Panel B: *Post-mortem*, the uterine artery was isolated. A segment was used for wire myography and another for stereology and histology.

Figure 2. MitoQ uptake in the mother, placenta and fetus. MitoQ uptake by maternal liver and placenta (A), and by fetal liver and fetal brain (B) at day 20 of gestation in normoxic (N), hypoxic (H), hypoxic+MitoQ (HQ) and normoxic+MitoQ (NQ) rodent pregnancy. N=7-10. *P < 0.05 *vs*. N (two-way ANOVA + Tukey Test).

Figure 3. Uterine artery vasoconstrictor function. Concentration response curves and area under the curve (AUC) (mean±S.E.M) for constrictor reactivity to norepinephrine (NE, A), Angiotensin II (B) and the protein kinase C activator PDBu (C) in uterine arteries isolated from normoxic (N), hypoxic (H), hypoxic+MitoQ (HQ) and normoxic+MitoQ (NQ) rodent pregnancy. N=7-9. *P < 0.05 *vs*. N; † P < 0.05 *vs*. H (two-way ANOVA + Tukey Test).

Figure 4. Uterine artery vasodilator function. Concentration response curves and area under curves (AUC) (mean±S.E.M) for reactivity to nitroprusside (SNP), acetyl-choline (ACh) and

744 the Ca^{2+} activated K^{+} channel BKCa activator NS1619 in uterine arteries isolated from
745 normoxic (N, white), hypoxic (H, grey), hypoxic+MitoQ (HQ, red) and normoxic+MitoQ (NQ,
746 blue) rodent pregnancy. N=7-11. *P< 0.05 *vs*. N; † P<0.05 *vs.* H (two-way ANOVA + Tukey
747 Test). In Panel D, the positive SEM above the white histogram is for the total AUC. The
748 negative SEM within the white histogram is for the NO-independent component. The positive
749 SEM above the black histogram is for the NO-dependent component.

750 **Figure 5. Uterine artery stereology and histology.** Data are mean ± S.E.M for wall thickness
751 (A), wall/lumen area ratio (B), and the wall and lumen areas expressed as a percentage of the
752 total vessel area (Relative Wall and Lumen Areas, C) for uterine arteries isolated from
753 normoxic (N), hypoxic (H), hypoxic+MitoQ (HQ) and normoxic+MitoQ (NQ) rodent
754 pregnancy. Scaled representative examples of light micrographs are shown in (D).
755 Magnification for micrographs × 20. Bar represents 100 μm. *P<0.05 *vs*. N (Generalized linear
756 model). For Panel C, the * above the white histogram represents a significant difference in the
757 white compartmental histogram between N and H. For Panel C, the * above the black
758 histogram represents a significant difference in the black compartmental histogram between N
759 and H.