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Selenium supplementation induces mitochondrial biogenesis in trophoblasts

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ABSTRACT

Introduction: Placental oxidative stress has been implicated in pregnancy complications and previous work has shown that selenium can protect trophoblast mitochondria from oxidative stress. This report examines mitochondrial function and content in trophoblasts supplemented with selenium.
Methods: Swan-71, JEG-3 and BeWo cells and placental tissue were incubated with sodium selenite or selenomethionine. Mitochondrial function was examined in a respirometer. Mitochondrial content was determined using RT-PCR. The levels of the mitochondrial biogenesis markers selenoprotein H, PGC-1α and NRF-1 was examined by western blotting.
Results: Mitochondrial respiration was significantly enhanced post selenium supplementation in cells and tissues. Selenium supplementation increased mitochondrial content and up-regulated mitochondrial biogenesis mediators in cells.
Discussion: These results emphasise the importance of selenium in mitochondrial regeneration in trophoblasts.

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1. Introduction

Placental oxidative stress is important in the aetiology of complications of pregnancy such as preeclampsia [1,2] and pre-term labour [3]. Oxidative stress describes an imbalance between pro-oxidants such as reactive oxygen and nitrogen species (RONS) and anti-oxidants. Many anti-oxidants are present in our diet, and they are important in maintaining redox balance in all cells. However, they work at a 1:1 stoichiometry and are limited in the amount of RONS they can neutralize. Enzymatic anti-oxidants generally feature very high turnover rates, provide the ability to limit damaging effects of RONS, and are present in all cells [4].

The glutathione and thioredoxin systems are key components of the anti-oxidant network that provide such protection. Two members of these systems, glutathione peroxidase (GPx) and thioredoxin reductase (TrxR) are seleno-proteins. They contain selenocysteine at their active site and are devoid of enzymatic activity in its absence [5]. The expression and activity of these enzymes is dependent on an adequate supply of either inorganic or organic selenium. For many years our research has focussed on the importance of these enzymes in cellular and animal models and exploring how an adequate selenium supply is correlated to initiation/progression of pathologies such a cardiovascular diseases [6] and complications of pregnancy [7,8].

Mitochondria are central in the development of oxidative stress and cell survival is ultimately dependent on how mitochondria are able to counteract the production of RONS. This is a natural consequence of oxygen use during respiration, which is elevated during chronic hypoxia or during periods of ischemia and reperfusion [9]. Any perturbation to the flow of electrons through the electron transport chain or the provision of oxygen as the final electron acceptor exacerbates electron leakage and results in the generation of partially reduced forms of oxygen and nitrogen, which constitute RONS. In this context, we have previously investigated selenium supplementation as a means to increase anti-oxidant defences and thereby improve mitochondrial resilience to oxidative insult in the cause of pathologies characterised by enhanced oxidative stress, such as preeclampsia [7,10].

Stressed mitochondria are able to return to normal function

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through anti-oxidant defences, by selectively removing damaged macromolecules and undergoing an elegant process of fusion and subsequent fission [11]. If recovery is not possible, damaged mitochondria will be removed by autophagy, or if the mitochondrial dysfunction is severe and widespread then the release of Cytochrome C will activate the intrinsic pathway to apoptosis and cell death [12]. This series of events is critical to cell turnover in the human placenta where mitochondrial oxidative stress has been shown to drive shedding of placental debris into the maternal circulation which may then elicit a hostile maternal immune response and endothelial cell activation which underpin the pathophysiology of disease such as preeclampsia [13,14].

In previous investigations we have shown that selenium supplementation can protect trophoblasts from mitochondrial stress through the up regulation of anti-oxidant systems [7]. We hypothesized that selenium might also be improving cellular function by regulating mitochondrial biogenesis, possibly through the recently characterised selenoprotein H (Sel H), which has the capacity to up-regulate key components of the mitochondrial biogenesis pathways [15]. In the present study we aimed to investigate at what point/s in the electron transport system selenium had its effect, and to determine if the improvement in mitochondrial function was due to an increase in mitochondrial content, and if this might be regulated through Sel H. As trophoblasts form the feto–maternal interface and are critical in the transport of nutrients and oxygen to the developing fetus, mitochondrial content and functionality was examined in three trophoblast-like cell lines, and we have extended some of our observations into explants of placental tissues from first trimester pregnancies.

2. Material and methods

2.1. Cell culture

Culture of Swan-71, BeWo and JEG-3 cell lines was as previously described [7]. Cells were supplemented with 100 nM sodium selenite (Sigma, Australia) or 500 nM selenomethionine (ICN Chemicals, Australia) for 24 h.

2.2. Measurement of mitochondrial respiration in trophoblast-like cell lines

Mitochondrial respiration was measured in an Oxygraph-2k (Oroboros Instruments, Austria). Trophoblast-like cell lines were tested for routine respiration and respiration of different mitochondrial complexes as detailed in Supplementary Information.

2.3. qPCR for determination of mtDNA content

DNA was extracted using the Genomic DNA Purification Kit (Thermo Scientific, Australia), and DNA concentration was measured on the 2000c Nanodrop (Thermo Scientific) according to manufacturers' protocols. 25 ng/μL of DNA was used in qPCR, and assessed with 5x Eva Green qPCR SuperMix-UDG (Solis BioDyne, Australia) on an Eco qPCR System (Illumina, Australia) using PCR conditions 95 °C 15 min, (95 °C 15 s, 60 °C 20 s, 72 °C 20 s for 40 cycles), followed by melt curve analysis [16]. The level of nDNA and mtDNA genes was evaluated using primers specified in Table 1, and the mtDNA/nDNA ratio was calculated.

2.4. Western blot

Western blot for selenoprotein H (Sel H), peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α), nuclear respiratory factor 1 (NRF-1), and β-actin was carried out using well-established methods, as detailed in Supplementary Information.

2.5. Tissue collection and culture

This study was approved by the Auckland Regional Ethics Committee, New Zealand, and all tissues were obtained following written informed consent. Placental tissue was obtained from 19 pregnancies (7.2–13.0 weeks of gestation) following elective surgical termination. Tissue was collected within 10 min of delivery, rinsed briefly in PBS, immediately transferred to HTK transplant solution (Essential Pharmaceuticals LLC, USA) on ice and transported to the laboratory. Placental explants of 10–40 mg wet weight were cultured at 37 °C in a humidified ambient oxygen atmosphere with 5% CO_2 in Dulbecco's modified Eagle's medium salts/F12 (Invitrogen, New Zealand) containing 10% fetal bovine serum, 5 ng/mL epidermal growth factor, 5 μg/mL insulin, 10 μg/mL transferrin, 400 U/L human chorionic gonadotrophin, 100 μg/mL streptomycin and 100 U/L penicillin. Matched explants of villous tissue were treated with selenium (NaSe 100 nM) or PBS vehicle control in culture for 4, 12, 14, 24, 48 or 96 h.

2.6. Measurement of mitochondrial respiration in tissue

Mitochondrialrespiration was measured in an Oxygraph-2k in matched explants of villous placenta supplemented with or without 100 nM sodium selenate for 4, 12, 14, 24, 48 or 96 h, and measurements were also made at time zero, defined as the time tissues arrived at the laboratory, as detailed in Supplementary Information. At each time point the number of placentae investigated were 4 h n = 11, 12 h n = 5, 14 h n = 6, 24 h n = 4, 48 h n = 5, 96 h n = 4.

2.7. Statistical analysis

All the data are presented as mean ± SD. Statistical analysis was performed using Graph Pad, PRISM version 6 (GraphPad, USA). A Grubbs' test was used to determine significant outlier values and data were tested for normality. To analyse differences between treatments, a Kruskal–Wallis test with Dunn's multiple comparison test was used in Fig. 1A and Fig. 2, and a two-tailed Wilcoxon

Table 1
Real time PCR primers.

Genes	Target	Forward primer	Reverse primer
MTRT1	mtDNA	CAC CCA AGA ACA GGG TTT GT	TGG CCA TGG GTA TGT TGT TA
MTRT2	mtDNA	TCC TCC TAT CCC TCA ACC CC	CAC AAT CTG ATG TTT TGG TTA AAC
MTRT3	mtDNA	CAT CTG GTT CCT ACT TCA GGG	TGA GTG GTT AAT AGG GTG ATA GA
MTRT4	mtDNA	ATG GCC CAC CAT AAT TAC CC	CAT TTT GGT TCT CAG GGT TTG
MTB2M	nDNA	TGC TGT CTC CAT GTT TGA TGT ATCT	TCT CTG CTC CCC ACC TCT AAG T
MTBA	nDNA	AGC GGG AAA TCG TGC GTG AC	AGG CAG CTC GTA GCT CTT CTC

mtDNA = mitochondrial DNA; nDNA = nuclear DNA.

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