Glucocorticoids dysregulate cellular viability, mitochondrial membrane potential and cellular respiration differently in normal compared to high glucose environments

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Maternal stress is known to impair placental development, alter birth weight and program chronic disease in offspring. Furthermore, stress is known to increase the risk of developing gestational diabetes mellitus, a common and important complication of pregnancy. I have previously performed a range of animal studies to elucidate the mechanistic pathways which relate maternal stress to impaired physiology in offspring. These studies have demonstrated a key role of the placenta in mediating offspring disease. Administration of either the synthetic glucocorticoid dexamethasone or the endogenous glucocorticoid corticosterone in mice altered glucocorticoid related pathways as well as o-linked glycosylation and glycogen storage in the placenta. This suggests an important interaction between glucocorticoids and glucose mediated pathways in placental dysfunction and programmed disease. In addition, I have recently demonstrated that glucocorticoid administration alters placental oxidative stress and reduces mitochondrial number. However, the role of glucocorticoids in mediating placental mitochondrial function remains poorly understood. This study aimed to investigate mitochondrial respiration following cortisol exposure (the endogenous glucocorticoid in humans) in placental cells (trophoblasts) exposed normal or high glucose conditions.

Swan 71 trophoblast cells were seeded into 96 well plates or T75 flasks and exposed to one of six treatment conditions. 1- normal glucose (5mmol), no cortisol, 2- normal glucose, 100nM cortisol, 3- normal glucose, 1000nM cortisol, 4-high glucose (17mmol), no cortisol, 5-high glucose, 100nM cortisol and 6-high glucose, 1000nM cortisol. After 24 hours of treatment, cells were assayed for viability using a 3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) assay or assayed for membrane potential by measuring Safranin O uptake following digitonin permeabilisation. Mitochondrial respiration was assessed using an oxygraph 2k (OROBOROS Instruments).

Under normal glucose conditions, both 100nM and 1000nM of cortisol increased cellular viability whereas cell viability was not affected by cortisol under high glucose conditions. Cells exposed to high glucose conditions however had increased viability compared to cells treated with normal glucose. Under normal glucose conditions, mitochondrial membrane potential was increased by 100nM of cortisol but reduced by 1000nM of cortisol. Whereas under high glucose conditions, both concentrations of cortisol increased mitochondrial membrane potential. Control cells exposed to high glucose had lower membrane potential than cells exposed to normal glucose. Routine respiration, leak respiration, maximum respiratory capacity and spare respiratory capacity were all unaffected by cortisol when grown in normal glucose media. In contrast, routine respiration, leak respiration and maximum respiratory capacity were all reduced by both doses of cortisol when grown in high glucose conditions. Spare respiratory capacity was not affected by cortisol in high glucose conditions.

This study highlights that the effects of glucocorticoids on trophoblast function depends on glucose availability. Under normal glucose conditions, cortisol stimulates growth/viability but has dose dependant effects on mitochondrial membrane potential while having no effect on mitochondrial respiration. In contrast, cortisol had no effect on cell growth/viability in high glucose conditions but increases mitochondrial membrane potential and reduces respiration. This is particularly relevant given my previous findings that glucocorticoids increase glucose storage within the placenta and that placental oxidative stress may play a role in the pathophysiology of programmed offspring disease. Furthermore, this may provide insight into the role of glucocorticoids in gestational disorders where glucose levels and stress are known to be contributing factors, such as gestational diabetes mellitus.