Abstract

In humans, low birth weight and abnormal postnatal growth are associated with higher risk of developing adult diseases, including insulin resistance, type 2 diabetes and hypertension. Although maternal undernutrition accounts for the majority of low birth weight cases in developing countries, uteroplacental insufficiency resulting in impaired placental perfusion and poor fetal nutrition is primarily responsible for instances of fetal growth restriction in the Western world. Rats exposed to uteroplacental insufficiency are used as a model since they also display metabolic disturbances and elevated blood pressure in adulthood. Skeletal muscle insulin resistance is a major contributor to whole body insulin resistance as it is responsible for at least 80% of the body’s insulin stimulated glucose uptake. Part of the skeletal muscle deficit that may contribute to insulin resistance is impaired mitochondrial biogenesis, which is the synthesis of new mitochondria. Mitochondria are organelles found in most cells of the body and their primary role is to synthesise ATP through substrate oxidation. Increased intramuscular lipids can be associated with reduced mitochondrial biogenesis and impaired insulin signalling. Previous studies from our laboratory have shown that male rats exposed to uteroplacental insufficiency (Restricted) or a reduction in litter size (Reduced) after birth, have abnormal postnatal growth and up to 50% reductions in skeletal muscle gene and protein markers of mitochondrial biogenesis (PGC-1α, Tfam, COX IV) compared with Controls at 6 months of age.

A major aim of Chapter 2 was to determine whether these skeletal muscle mitochondrial biogenesis deficits in Restricted adult offspring manifest in late gestation and early postnatal life. Whether key genes involved in skeletal muscle development (Myogenic regulatory factors: MyoD, myogenin, MRF4 and IGF-1) were also affected by uteroplacental insufficiency was investigated. A second aim of Chapter 2 was to determine whether improving early postnatal nutrition, through cross-fostering Restricted pups one day after birth onto mothers with better milk nutrition, could improve early growth and normalise skeletal muscle gene expression of mitochondrial biogenesis markers and myogenic regulatory and growth factors. In both studies the impact of uteroplacental insufficiency and cross-fostering was investigated in males and females separately. Uteroplacental insufficiency was induced in Wistar Kyoto rats through bilateral uterine vessel ligation surgery at 18 days gestation yielding Restricted males and females that were compared with sham operated Controls and were investigated at gestational day 20 (E20) postnatal days 1 (PN1), 7 (PN7) and 35 (PN35) (n=8-10/group). A separate cohort of Restricted and Control offspring were cross-fostered onto a different Control or Restricted mother 1 day after birth and killed at day 7 (n=6-10/group). Findings from Chapter 2 demonstrated that mitochondrial biogenesis genes were largely intact following uteroplacental insufficiency and developmental age was a major factor regulating skeletal muscle mitochondrial and developmental genes. Uteroplacental insufficiency however, increased IGF-1 mRNA by ~50% (P<0.05) in male offspring at E20 and PN1 and in females increased MyoD mRNA by ~40% (P<0.05) at PN7, perhaps indicative of delayed myogenesis and compensatory upregulation in the face of nutrient restriction. Despite, uteroplacental insufficiency having minor impact on mitochondrial biogenesis, cross-fostering in the first 7 days of postnatal life increased Restricted offspring skeletal muscle COX IV expression up to 50% higher than Controls (P<0.05). Whether these subtle genes effects are sustained into adulthood and impact on later skeletal muscle phenotype is
unknown. It therefore appears that reductions in adult mitochondrial biogenesis markers previously observed in Restricted offspring likely develop after weaning.

Exercise training is well known to increase skeletal muscle mitochondrial biogenesis, improve insulin sensitivity and reduce blood pressure. Previous studies have shown that exercise training in normal birth weight rats early in life could prevent diet induced obesity for up to 10 weeks after exercise cessation, which may be a ‘reprogramming’ effect of early exercise. Therefore a major aim of this thesis was to determine whether early life exercise training (5-9 weeks) could normalise adult skeletal muscle mitochondrial biogenesis and offset metabolic and cardiovascular disease in Restricted offspring in later life (Chapters 3-5). Another major aim of this thesis was to investigate whether later life exercise training (20-24 weeks) in Restricted offspring could yield normal exercise-induced adaptations and thus reduce disease progression in adulthood. Since normal birth weight male offspring that had their litter size reduced after birth, to match that of Restricted litters, also demonstrate abnormal postnatal growth trajectories and develop adult disease, Reduced litter offspring were also investigated. Finally, since the adult disease phenotype is more pronounced in males than females, the impact of exercise training was examined in males only. Exercise training involved treadmill running for 60 minutes a day at 20m/minute for 5 days/week (n=10/group) from 5-9 weeks or 20-24 weeks of age with post mortem at 9 or 24 weeks. We hypothesised that early life exercise training would ‘reprogram’ Restricted and Reduced offspring to improve later life skeletal muscle mitochondrial biogenesis and offset the development of insulin resistance and high blood pressure.

Chapter 3 determined the impact of early and later exercise training on skeletal muscle mitochondrial biogenesis and intramuscular lipids in Restricted and Reduced offspring. Consistent with previous studies, Restricted and Reduced offspring skeletal muscle PGC-1α protein expression was reduced by ~20% (P<0.05) at 24 weeks of age and was accompanied by up to 4-fold higher levels of intramuscular lipids (P<0.05). Restricted and Reduced offspring responded to early and later exercise with normal skeletal muscle adaptations, including increased mitochondrial biogenesis protein markers and mitochondrial enzyme activities (P<0.05). However, contrary to our hypothesis, early exercise training did not ‘reprogram’ the skeletal muscle to normalise mitochondrial biogenesis in later life. Importantly, later exercise training was effective in increasing PGC-1α protein in Restricted and Reduced offspring (P<0.05) to levels comparable to exercised Controls and suggests that later exercise can be used as an effective therapeutic intervention to ensure a healthy progression into adulthood for humans born small.

Chapter 4 determined whether early life exercise could improve later insulin sensitivity in Restricted and Reduced offspring, assessed by an intraperitoneal glucose tolerance test (IPGTT). Adult sedentary Restricted and Reduced offspring demonstrated elevated HOMA-IR (P<0.05), by ~65% and 80%, respectively, indicative of hepatic insulin resistance. Considering the lack of a ‘reprogramming’ effect of early exercise on skeletal muscle mitochondrial biogenesis in later life (Chapter 3), it was not surprising that following early life exercise, the response to IPGTT was similar to sedentary
offspring. Surprisingly, however, early exercise provided subtle metabolic improvement to adult Reduced offspring with lower peak plasma insulin concentration during IPGTT by ~30% despite no skeletal muscle exercise adaptations remaining. Later exercise training improved insulin sensitivity in Controls \((P=0.05)\) and tended to improve insulin sensitivity in Reduced \((P=0.07)\) offspring, which displayed reduced second phase insulin secretion during IPGTT. Since Restricted offspring increased skeletal muscle mitochondrial biogenesis following later exercise, but did not display improved insulin sensitivity, it appears that there is a divergence between mitochondrial biogenesis and insulin sensitivity in Restricted offspring. However, later exercise was associated with a tendency for increased first phase insulin secretion in Restricted offspring \((P=0.07)\) suggesting improved pancreatic response to glucose and improved glucose tolerance.

Unlike previously published results that reported elevated blood pressure in male Restricted and Reduced offspring at 6 months of age, Chapter 5 found that in the current animal cohort, systolic blood pressure was not elevated in Restricted and Reduced offspring at 24 weeks of age and there was no change with early or later exercise intervention. Surprisingly, early life exercise training increased whole heart weight in Control, Restricted and Reduced offspring by ~8% at 24 weeks of age \((P<0.05)\), despite no exercise being performed between 9 and 24 weeks. This magnitude of change (~8%) is likely to be physiologically relevant and be accompanied by functional improvements, especially since the increase in heart weight was not accompanied by changes in blood pressure and may help to reduce the risk of cardiovascular disease development in Restricted or Reduced offspring at an older age.

In conclusion, this thesis has provided important evidence to suggest that skeletal muscle mitochondrial biogenesis is largely intact from late gestation to at least 9 weeks of age in offspring exposed to uteroplacental insufficiency. It therefore appears that the deficits in mitochondrial biogenesis observed in adulthood develop after 9 weeks of age. This thesis has also demonstrated organ specific susceptibility to the ‘reprogramming’ effects of early life exercise such that early exercise training had no ‘reprogramming’ effect to prevent impaired skeletal muscle mitochondrial biogenesis and insulin sensitivity and secretion in adulthood, but appeared to provide long-term benefits for heart size. Later exercise training elicited normal adaptations in Restricted and Reduced offspring and would likely, if maintained, provide beneficial effects in offspring predisposed to developing metabolic and cardiovascular disease. Exercise training is a readily translatable intervention to humans and the results of this thesis provide the rationale for clinical studies and preventative intervention strategies that will be instrumental to preserve metabolic and cardiovascular function in humans predisposed to later disease and ensure a healthy start to life in human babies born small.