Does folic acid supplementation during pregnancy reduce skeletal muscle mitochondrial biogenesis and insulin signalling in adult offspring?

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Background: Folic acid plays a significant role in the health of a foetus and is often supplemented to women during pregnancy to prevent neural tube defects such as *Spina Bifida* (Lucock & Yates, 2009). However, recent evidence suggests that high doses of folic acid supplementation (FAS) during pregnancy might permanently alter the skeletal muscle metabolism in adult offspring. Indeed, Owens *et al.* (2009) have found that high FAS during pregnancy increases insulin sensitivity in adult males but decreases insulin sensitivity in adult female offspring (Owens *et al.*, 2009). Mitochondria are crucial to skeletal muscle metabolism and impaired mitochondrial biogenesis (synthesis) is implicated in the aetiology of insulin resistance and type 2 diabetes (Liu *et al.*, 2009). Interestingly, Owens *et al.* also found decreased expression of genes involved in mitochondrial biogenesis, such as PGC-1α, and genes involved in insulin signalling, such as Akt in the skeletal muscle of these FAS animals (Owens *et al.*, 2009). Therefore, it is possible that high dose folic acid supplementation during pregnancy could also down-regulate the protein expression of skeletal muscle mitochondrial biogenesis markers and insulin signalling proteins in the offspring.

Aims: To examine the long-term effects of high folic acid supplementation during pregnancy on the markers of mitochondrial biogenesis and insulin signalling proteins in the skeletal muscle of the adult offspring.

Methods: Pregnant rats received either a control diet containing a normal amount of folic acid (2mg/kg) or a FAS diet containing a high dose of folic acid (6mg/kg). At 90 days of age, the young adult offspring were given intraperitoneal injection of sodium pentobarbital (180 mg/kg body weight) and the skeletal muscle was dissected out and quickly frozen in LN_2 and stored at −80°C. Skeletal muscle was collected and markers of mitochondrial biogenesis, the levels of mitochondrial proteins and insulin signalling proteins were measured by western blotting.

Results: IRS-1 protein expression was 42% higher in male FAS offspring compared to the male Control offspring ($p<0.05$). The protein abundance of other insulin signalling proteins, such as Akt was not altered in either sex following FAS. Furthermore, there were no other significant differences between FAS offspring and Control offspring in either sex for markers of mitochondrial biogenesis (PGC-1α and Tfam) and the levels of mitochondrial proteins (COX IV and Cytochrome C).

Conclusions: The results indicate that young adult males whose mothers were exposed to high folate levels during pregnancy had increased expression of the insulin signalling protein IRS-1 in association with improved insulin sensitivity that has been previously reported (Owens *et al.*, 2009). However, exposure to high levels of folate during pregnancy does not appear to alter markers of mitochondrial biogenesis or mitochondrial proteins in the adult offspring of either male or female rats.

Liu, J, Shen, W, Zhao, B, Wang, Y, Wertz, K, Weber, P & Zhang, P (2009) Targeting mitochondrial biogenesis for preventing and treating insulin resistance in diabetes and obesity: Hope from natural mitochondrial nutrients. *Advanced Drug Delivery Reviews,* **61,** 1343-52.

Lucock, M & Yates, Z (2009) Folic acid fortification: a double-edged sword. *Current Opinion in Clinical Nutrition and Metabolic Care,* **12,** 555-64.

Owens, J, Kong, W, Grant, P, Moretta, S, De Blasio, M, Harland, M, Gatford, K, Robinson, J & Dziadek, M (2009) Maternal folic acid supplementation in the rat improves insulin sensitivity in adult male offspring but impairs that of female offspring. *Proceedings of the Perinatal Society of Australia and New Zealand meeting in Darwin, April 2009* 45.