Inorganic nitrate supplementation enhances skeletal muscle insulin sensitivity in nNOS null mice

M. Zhang,[1] J.P. Kerris,[1] F. Falcão-Tebas,[1] E.C. Marin,[1] S. Andrikopoulos[2] and G.K. McConell,[1] [1]Clinical Exercise Science Program, Institute of Sport, Exercise and Active Living (ISEAL), Victoria University, Melbourne, VIC 8001, Australia and [2]Islet Biology and Metabolism Laboratory, Department of Medicine, The University of Melbourne, Melbourne, VIC 8001, Australia.

Physiological concentrations of nitric oxide (NO) are essential for normal skeletal muscle and endothelial function. People with type 2 diabetes have decreased skeletal muscle neuronal NO synthase (nNOS) expression. NO can also be formed from nitrite (NO_2) and nitrate (NO_3), which is found in high quantities in green leafy vegetables. Therefore, NO_2 and NO_3 can provide an alternative source of NO which may be of great importance. Several studies have recently shown NO_3 supplementation improves disturbed glucose metabolism in endothelial NOS (eNOS) null mice (Carlström *et al.*, 2010) and exhibit anti-obesity and anti-diabetic effects in ob/ob mice, C57BL/6J mice and rats (Roberts *et al.*, 2015). Long term (17 months) dietary nitrate supplementation in C57BL/6J mice improves fasting insulin and insulin response (Hezel *et al.*, 2015).

In our study, dietary supplementation of NO_3 at 1mM (Carlström *et al.*, 2010) in drinking water was given for 14 weeks to male and female nNOS null mice which are insulin resistant (Huang *et al.*, 1993; Shankar *et al.*, 2000). IpGTT, IpITT, *ex vivo* muscle insulin stimulation and muscle mitochondria function were examined. After 14 weeks, overnight-fasted mice were anesthetised with pentobarbital sodium (60 mg/kg intraperitoneal) and both *extensor digitorum longus* (EDL) and *soleus* muscles were rapidly dissected for insulin-stimulated glucose uptake *ex vivo, gastrocnemius* muscles were dissected for mitochondria respiration analysis and brown and white fat, liver, *tibialis anterior* (TA) muscles and serum were collected for further analysis. We found chronic nitrate treatment lowered overnight fasting glucose levels in males (4.2 mM *vs* 4.9 mM, P=0.02) and improved glucose tolerance in females (P=0.0001-0.02). In both genders, NO_3 treatment enhanced EDL skeletal muscle insulin-stimulated glucose uptake (5.3-fold increase in NO_3 treatment group compared with 3.2 fold increase in vehicle group, $P \leq 0.01$). There was no effect of NO_3 treatment on insulin-stimulated glucose uptake in the *soleus*. Chronic nitrate treatment had no effect on body weight, caloric intake, fat composition or skeletal muscle mitochondria function in any group.

In conclusion, these results suggest that nitrate supplementation may increase flux through the nitrate–nitrite–NO pathway which partly compensates for a lack of skeletal muscle endogenous NO generation by nNOS.

Carlström M, Larsen FJ, Nyström T, Hezel M, Borniquel S, Weitzberg E, Lundberg JO. (2010). Dietary inorganic nitrate reverses features of metabolic syndrome in endothelial nitric oxide synthase-deficient mice. *Proc Natl Acad Sci USA* **107**, 17716-20.

Roberts LD, Ashmore T, Kotwica AO, Murfitt SA, Fernandez BO, Feelisch M, Murray AJ, Griffin JL. (2015) Inorganic nitrate promotes the browning of white adipose tissue through the nitrate-nitrite-nitric oxide pathway. *Diabetes* **64**, 471–484.

Hezel MP, Liu M, Schiffer TA, Larsen FJ, Checa A, Wheelock CE, Carlström M, Lundberg JO, Weitzberg E. (2015) Effects of long-term dietary nitrate supplementation in mice. *Redox Biol* **29**, 234-242.

Huang PL, Dawson TM, Bredt DS, Snyder SH, Fishman MC. (1993) Targeted disruption of the neuronal nitric oxide synthase gene. *Cell* **75**, 1273–1286.

Shankar RR, Wu Y, Shen HQ, Zhu JS, Baron AD. (2000). Mice with gene disruption of both endothelial and neuronal nitric oxide synthase exhibit insulin resistance. *Diabetes* **49**, 684-687.

Acknowledgements: Australian Institute for Musculoskeletal Science (AIMSS) Grant 2014 to Dr. Mary Zhang.