Role of nitric oxide in mitochondrial biogenesis in L6 myocytes

G.P.Y. Ng,[1] M. Phillips,[1] L. Macaulay,[2] G.D. Wadley[1] and G.K. McConell,[1] [1]Department of Physiology, University of Melbourne, Parkville, VIC 3010, Australia and [2]Obesity and Diabetes Treatment Laboratory, CSIRO, Parkville, VIC 3010, Australia.

Background and Aim. Skeletal muscle mitochondrial biogenesis is reduced in people with type 2 diabetes and exercise increases mitochondrial biogenesis. The signalling mechanisms associated with this increase in mitochondrial biogenesis by exercise are not fully understood. Contraction is associated with increases in calcium levels, adenosine monophosphate activated protein kinase (AMPK) activity and nitric oxide (NO) production, and there is evidence that all three may play a role in skeletal muscle mitochondrial biogenesis (Ojuka, 2004; Nisoli *et al.*, 2004). For instance, caffeine, which causes sarcoplasmic reticulum calcium release, and 5-Aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR), an AMPK activator, both increase mitochondrial biogenesis in L6 myotubes (Ojuka, 2004). Neuronal NO synthase (nNOS) and endothelial NO synthase (eNOS) are expressed in muscle, and it has been shown that NO donors increase mitochondrial biogenesis in L6 myotubes (Nisoli *et al.*, 2004). Since nNOS and eNOS are calcium dependent enzymes, and AMPK phosphorylates nNOS and eNOS, it is possible that AICAR and caffeine increase mitochondrial biogenesis, at least in part, *via* NO. Our aim was to determine whether caffeine and AICAR increase mitochondrial biogenesis in L6 myotubes, at least in part, *via* NOS, by using the NOS inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME).

Methods. L6 rat myotubes cells were cultured and differentiated in DMEM media with 2% horse serum (HS), 1% penicillin and streptomycin, on a 6-well plate coated with type I collagen at 37°C. 8 different treatments were presented: No treatment; 100μM L-NAME; 100μM S-nitroso-N-acetylpenicillamine (SNAP, NO donor) ± 100μM L-NAME; 2mM AICAR ± 100μM L-NAME; or 5mM caffeine ± 100μM L-NAME. All treatments were applied for 5 hours per day over 5 days in a DMEM media containing 10% HS, 5% FBS, 1% BSA, 1% Penicillin and Streptomycin, 1mM L-carnitine and 0.5mM oleic acid. Total protein was extracted 24 hours following the final day of treatment. Protein abundance of PGC-1α, COX I and COX IV relative to β-actin was determined *via* immunoblotting using commercially available antibodies and fluorescence. The experiment was repeated 3 times on 3 different occasions ending up with n=8 for each treatment.

Results. AICAR, caffeine and SNAP treatments increased PGC-1α protein expression by approximately 1.9-fold ($p < 0.01$), 1.8-fold ($p < 0.01$) and 1.9-fold ($p < 0.01$) compared to control cells respectively. COX IV protein expression was also significantly upregulated in all treatments with a 3-fold ($p < 0.05$), a 2.5-fold ($p < 0.05$) and a 3.3-fold ($p < 0.05$) increase following AICAR, caffeine and SNAP treatments, respectively. Furthermore, COX I protein significantly increased with AICAR and SNAP treatments but not with caffeine treatment. As expected, the increase in PGC-1α, COX I and COX IV protein in response to SNAP were not significantly reduced by NOS inhibition. However, NOS inhibition caused a small attenuation of the increase in PGC-1α, COX I and COX IV protein expression in response to AICAR and caffeine such that the increases were no longer significant.

Conclusion. Our data suggest that NO induces the expression of the proteins PGC-1α, COX I and COX IV in L6 myocytes, consistent with previously demonstrated activation of mitochondrial biogenesis in these cells (Nilsoli *et al.*, 2003). We found some evidence that AICAR and caffeine may both be activating mitochondrial biogenesis, at least in part, *via* NO. However, further investigations are required to fully understand these cellular interactions.

Ojuka, E.O. (2004) *The Proceedings of the Nutrition Society* **63**, 275-8.

Nisoli, E. Falcome, S.., Tonello, C., Cozzi, V., Palomba, L., Fiorani, M., Pisconti, A., Brunelli, S., Francolini, M., Francolini, M., Cantoni, O., Cattuba, MO. Moncada, S. & Clementi, E. (2004) *Proceedings of the National Academy of Sciences USA* **101**, 16507-16512.