Effects of simvastatin on skeletal muscle mitochondrial biogenesis and mitochondrial enzyme activities

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Cardiovascular Disease affects 3.5 million Australians, with elevated levels of low density lipoprotein (LDL) cholesterol being a major risk factor. Statins, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, inhibit the rate limiting step in the mevalonate pathway and thus reduce cholesterol synthesis. Statins are the most prescribed drug in the world, with over 100 million prescriptions filled in 2004, in USA alone (Dirks *et al*., 2005). Although generally well tolerated, statins, particularly at high doses, can result in a skeletal muscle myopathy that ranges from muscle pain, stiffness, cramps and fatigue to rare cases of severe rhabdomyolysis. These events may affect up to 11% of patients (Bruckert *et al*., 2005). Preliminary evidence suggests that statin treatment in humans results in decreased skeletal muscle mitochondrial enzyme activity (Paiva *et al.*, 2005). Moreover, recent studies suggest that statins appear to preferentially affect fast twitch skeletal muscle fibre types (Westwood *et al*., 2005). Our aim was to investigate whether high dose statin administration would lead to an increase in mitochondrial biogenesis as a compensatory effect against decreased mitochondrial enzyme activity.

Male *Sprague Dawley* rats (5-6 weeks) were assigned to control (n=8), simvastatin 60 $mg \cdot kg^{-1} \cdot day^{-1}$ (n=8) and simvastatin 80 $mg \cdot kg^{-1} \cdot day^{-1}$ (n=8) treatment groups. 60 $mg \cdot kg^{-1} \cdot day^{-1}$ and 80 $mg \cdot kg^{-1} \cdot day^{-1}$ dosage was chosen as a previous study on rats was successful at inducing a statin associated myopathy after 10 days (Westwood *et al*., 2005). Rats were orally gavaged daily for 14 days with vehicle (5% methylcellulose) or vehicle + simvastatin (5 ml/kg). On day 15, rats were killed with an overdose of pentobarbitone (0.7ml pentobarbital sodium-325$mg \cdot ml^{-1}$) and the soleus, EDL and plantaris muscles were rapidly excised. Muscles were examined for activities of citrate synthase (CS) (citric acid cycle) and beta hydroxyacyl-coenzyme A dehydrogenase (β-HAD) (fatty acid beta oxidation pathway). A range of histological stains were utilised on soleus and plantaris (8μm sections) for examining general muscle morphology by haematoxylin + eosin (H+E). Stains indicating mitochondrial function including, succinate dehydrogenase (SDH), cytochrome c oxidase (COX), nicotinamide adenine dinucleotide tetrazolium reductase (NADH) and a combined stain of COX/ SDH were also utilised. Western blotting analysis of soleus, plantaris and EDL muscles were conducted to investigate mitochondrial biogenesis markers (PGC-1α, NRF-1 and T-fam). Upstream signalling pathways associated with mitochondrial biogenesis were also investigated (P-CREB, P-Akt, mTOR, P-AMPK, NOS, P-ACC).

The administration of simvastatin for 14 days did not alter *ad libitum* food consumption in either the 60 $mg \cdot kg^{-1} \cdot day^{-1}$ or 80 $mg \cdot kg^{-1} \cdot day^{-1}$ group ($p > 0.05$) compared to controls, however, normalised (to starting weight) body weights indicated that the 80 $mg \cdot kg^{-1} \cdot day^{-1}$ and 60 $mg \cdot kg^{-1} \cdot day^{-1}$ groups had 20.5% and 10.2% less weight gain, respectively, compared to control group ($p < 0.05$). Plantaris ($p < 0.05$) and EDL ($p < 0.01$) β-HAD activity was reduced in the 80 $mg \cdot kg^{-1} \cdot day^{-1}$ group. In addition, EDL β-HAD activity was reduced in the 60 $mg \cdot kg^{-1} \cdot day^{-1}$ group ($p < 0.05$). CS activity was significantly increased in plantaris 60 $mg \cdot kg^{-1} \cdot day^{-1}$($p < 0.05$) and 80 $mg \cdot kg^{-1} \cdot day^{-1}$ ($p < 0.01$) treatment groups. There was no change in β-HAD activity in soleus and no change in CS activity in soleus and EDL. Western blot analysis revealed no change in any mitochondrial biogenesis markers, or upstream protein phosphorylation involved in pathways responsible for the activation of mitochondrial biogenesis. Histological stains did not indicate altered mitochondrial SDH, NADH and COX and H+E did not reveal any abnormal morphology.

In conclusion, high dose statins induced a significant reduction in fatty acid beta oxidation enzyme function in the fast-twitch muscles but not in the slow-twitch soleus. This suggests fast-twitch muscles are more sensitive to statin administration compared to slow-twitch skeletal muscle. The administration of simvastatin had no effect on mitochondrial biogenesis markers or muscle morphology. These data show that although there was altered beta oxidation enzyme activity, it was not sufficient to cause an up regulation of mitochondrial biogenesis or alter muscle structure and function over a period of two weeks.

Bruckert E, Hayem G, Dejager S, Yau C, Begaud B. (2005). *Cardiovascular Drugs and Therapy* **19**: 403-14.

Dirks AJ and Jones KM (2006). *American Journal of Physiology- Cell Physiology* **291**: C1208-12.

Paiva H, Thelen K M, Van Coster R, Smet J, De Paepe B, Mattila KM, Laakso J, Lehtimaki T, Von Bergmann K, Lutjohann D, Laaksonen, R. (2005). *Clinical Pharmacology and Therapeutics* **78**: 60-8.

Westwood FR, Bigley A, Randall K, Marsden AM, Scott RC. (2005). *Toxicology and Applied Pathololology* **33**: 246-57.