The relative amounts of PTEN protein in rat cardiac and skeletal muscles and the effect of high dose statins

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The tumor suppressor PTEN (phosphatase and tensin homologue deleted from chromosome 10) is a dual protein and lipid phosphatase which negatively regulates the PI3K/AKT pathway thus affecting metabolism and growth. Muscle specific deletion of Pten enhances insulin-stimulated glucose uptake in mouse slow twitch soleus but not in fast twitch EDL muscle (Wijesekara *et al*., 2005). To date, few studies have examined PTEN protein expression in rat muscle and no studies have examined the relative expression of PTEN across muscles with different fiber type compositions and different functions. Two previous studies have shown that prolonged statin [3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) inhibitors] administration leads to increased PTEN protein levels in the heart (Planavila *et al*., 2008; Mensah *et al*., 2005) possibly via increased PPAR-γ mediated transcription (Teresi, *et al*., 2008). Others have shown that PPAR-γ agonists lead to reduced muscle PTEN levels (Kim *et al*., 2007). No studies have examined the effect of high dose statins on skeletal muscle PTEN protein expression. Therefore the aim of this study was to investigate: 1) the relative content of PTEN protein in cardiac and skeletal muscles of different fibre types and 2) the effect of prolonged high dose statin administration on PTEN protein expression in the tibialis anterior muscle.

Twenty four male Sprague Dawley rats (6-7 wks) were divided into three groups: 1) controls, 2) 60 $mg \cdot kg^{-1} \cdot d^{-1}$ simvastatin and 3) 80 $mg \cdot kg^{-1} \cdot d^{-1}$ simvastatin. All rats had *ad libitum* access to food and water. Rats were orally gavaged daily for 14 days with either a vehicle (0.5% methyl cellulose) or simvastatin + vehicle at a constant relative volume of $5ml \cdot kg^{-1}$ body mass. On day 15, rats were killed with an overdose of pentobarbitone (0.7ml of pentobarbital sodium-$325mg \cdot ml^{-1}$) and the heart and soleus, EDL, plantaris and tibialis anterior muscles rapidly dissected. Pten protein was analysed by western blot with the density of the PTEN protein band expressed relative to the amount of protein loaded onto each gel (60μg). Results are Mean ± SE. Statistical analysis performed with one-way ANOVA with Bonferoni post-test. Significance at $p < 0.05$.

Under control conditions, the PTEN antibody detected two protein bands in the heart samples suggestive of two different isoforms but only one band in all the skeletal muscles. The amount of PTEN protein in control muscles was greater ($p < 0.05$) in the heart (0.149 ± 0.009 A.U./μg protein; n = 8) compared to the skeletal muscles, with soleus (0.074 ± 0.002 A.U./μg protein) and tibialis anterior (0.089 ± 0.004 A.U./μg protein; n = 8) having greater amounts of PTEN than EDL (0.047 ± 0.007 A.U./μg protein; n = 8) and plantaris (0.047 ± 0.002 A.U./μg protein; n = 8) which were not different from each other ($p > 0.05$). Two weeks of either 60 $mg.kg^{-1} \cdot d^{-1}$ or 80 $mg \cdot kg^{-1} \cdot d^{-1}$ group resulted in 10.2% and 20.5% less body weight ($p>0.05$) gain compared to controls ($p < 0.05$). Neither 60 or 80 $mg \cdot kg^{-1} \cdot d^{-1}$ of simvastatin for 14 days resulted in a change in the amount of PTEN protein in the tibialis anterior muscle compared to Control muscles (0.368 ± 0.016 *vs* 0.361 ± 0.011 *vs* 0.410 ± 0.027 A.U./μg protein, respectively).

In conclusion, rat PTEN protein levels vary between cardiac and skeletal muscle and between skeletal muscles of different fibre types. In addition, in contrast to previous studies in cardiac muscle, prolonged statin administration did not alter skeletal muscle levels of PTEN protein.

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