The effect of taurine supplementation on taurine transporter content and ROS-induced lipid peroxidation during fatiguing contractions in rat skeletal muscle

C.A. Goodman,[1,2,4] D. Horvath,[3,4] C.G. Stathis,[3,4] K. Croft[5] and A. Hayes,[3,4] [1]School of Human Movement, Recreation and Performance, Victoria University, Melbourne, VIC 8001, Australia., [2]Department of Physiology, The University of Melbourne, Parkville, VIC 3010, Australia, [3]School of Biomedical and Health Sciences, Victoria University, Melbourne, VIC 8001, Australia, [4]Centre for Ageing, Rehabilitation, Exercise and Sport, Victoria University, Melbourne, VIC 8001, Australia and [5]School of Medicine and Pharmacology, University of Western Australia, Crawley, WA 6009, Australia.

Taurine (Tau; 2-aminoethane sulfonic acid), a non-toxic beta amino acid found in most mammalian cells, is reported to function as an osmotic regulator, a cell membrane stabilizer, a modulator of inflammation, an intracellular ion regulator (esp. calcium) and a direct or indirect antioxidant (for review see Huxtable, 1992). Studies in liver have shown that Tau may attenuate non-enzymatic reactive oxygen species (ROS)-induced lipid peroxidation (e.g. Yildirim *et al.*, 2007). It has also been shown in various cell lines and tissues that increased Tau levels down-regulates Tau transporter mRNA and protein expression (Tappaz, 2004). The aim of this study was to investigate: 1) whether Tau supplementation would increase muscle Tau content and lead to a decrease in Tau transporter protein; 2) whether continuous or repeated fatiguing tetanic contractions would lead to an increase in non-enzymatic ROS-induced lipid peroxidation as indicated by F_2-isoprostane production; and 3) whether increased muscle Tau can reduce any non-enzymatic ROS-induced lipid peroxidation during fatiguing repeated tetanic contractions.

Male Sprague Dawlay rats (8 wks) were supplemented with Tau in drinking water (2.5% w/v) for 2 weeks. Fast twitch *extensor digitorum longus* (EDL) muscles were dissected out under anaesthesia (Nembutal; 85mg/kg) in accordance with Victoria University AEEC procedures and subjected to one of two different stimulation protocols: 1) 10s continuous stimulation at a frequency of 100Hz (0.2ms pulse duration); 2) 3 min intermittent stimulation (1s stimulation at 100Hz followed by 4s recovery). Fatigued muscles and their non-fatigued contra-lateral controls were blotted, weighed, frozen in liquid N_2 and F_2-isoprostanes analysed by GC/MS (Mori *et al.*, 1999). Non-fatigued control muscles were also analysed for Tau content by HPLC and Tau transporter protein by western blotting.

Tau supplementation increased muscle Tau content by 39.5% (p = 0.0002, n=8) with no change in Tau transporter protein (p = 0.41, n=8). Ten seconds of continuous fatiguing tetanic stimulation, which reduced tetanic force by 60-66% of initial force, did not result in a change in the level of F_2-isoprostanes in either non-supplemented (n=8) or Tau supplemented muscles (n = 8) compared to their non-stimulated contra-lateral controls. After 3 min of intermittent stimulation, however, in which tetanic force was reduced by 90-92%, there was a significant main effect (p=0.0003; 2-way ANOVA with Bonferoni post-test) for contractions to increase F2-isoprostane levels by 46.7% (1.47 ± 0.09 *vs* 2.16 ± 0.13 pg F_2-isoprostanes/μg arachidonic acid; n = 8) and by 13.0 % (1.85 ± 0.13 *vs* 2.09 ± 0.09 pg F_2-isoprostanes/μg arachidonic acid; n = 8) compared to non-stimulated contra-lateral control muscles (n = 8). There was also a strong trend (p = 0.06) for Tau to attenuate F_2-isoprostane production than stimulated control muscles.

In conclusion, 2 wks of Tau supplementation significantly increased muscle Tau content but did not cause a down-regulation of Tau transporter protein expression. In addition, repeated tetanic contractions led to a significant increase in non-enzymatic ROS-induced lipid peroxidation, as indicated by raised F_2-isoprostane content. There was a strong trend for Tau to attenuate lipid peroxidation.

Huxtable RJ (1992) *Physiolgical Reviews*, **72**, 101-163.
Mori TA, Croft KD, Puddey IB, Beilin LJ. (1999) *Analytical Biochemistry*, **268**, 117-127.
Tappaz ML (2004) *Neurochemistry Research*, **29**, 83-96.
Yildirim Z, Kiliç N, Ozer C, Babul A, Take G, Erdogan D. (2007) *Annals of the New York Academy of Science*, **1100**, 553-561.