Altering the redox state of skeletal muscle by glutathione depletion increases the exercise-activation of PGC-1α

N.A. Strobel,[1,2] A. Matsumoto,[3] J.M. Peake,[4] S.A. Marsh,[5] T. Peternelj,[1] D. Briskey,[1] R.G. Fassett,[1] J.S. Coombes[1] and G.D. Wadley,[6,7] [1]School of Human Movement Studies, The University of Queensland, St Lucia, QLD 4072, Australia, [2]Office of the Pro-Vice-Chancellor (Health Advancement) Edith Cowan University, Joondalup, WA 6027, Australia, [3]Baker IDI Heart and Diabetes Institute, Melbourne, VIC 3004, Australia, [4]School of Biomedical Sciences, Queensland University of Technology, Brisbane, QLD 4059, Australia, [5]College of Pharmacy, Washington State University, Spokane, WA 99163, USA, [6]Department of Physiology, The University of Melbourne, Melbourne, VIC 3010, Australia and [7]School of Exercise and Nutrition Sciences, Deakin University, Burwood, VIC 3125, Australia.

A number of studies have attempted to elucidate the role of exercise-induced reactive oxidative species (ROS) in cell signalling and mitochondrial biogenesis (synthesis). Experimental approaches have included inhibiting ROS production, either by enzymatic inhibitors such as the administration of allopurinol, or through antioxidant supplementation (Gomez-Cabrera *et al.*, 2005; Wadley & McConell, 2010; Higashida *et al.*, 2011; Wadley *et al.*, 2013). An alternative approach is to exacerbate oxidative stress during exercise by reducing endogenous antioxidants. As such, we investigated the relationship between ROS, mitochondrial biogenesis, cell signalling and antioxidant enzymes by depleting skeletal muscle glutathione with diethyl maleate (DEM) resulting in increased oxidative stress during exercise. We hypothesized that by reducing intracellular glutathione, ROS would be increased with acute exercise, resulting in an increase in markers of mitochondrial biogenesis, cell signalling, and antioxidant enzymes.

Animals were divided into six groups: (1) sedentary control rats (n = 8); (2) sedentary rats treated with DEM (n = 8); (3) exercise control rats euthanized immediately after exercise (n = 8); (4) exercise rats + DEM (n = 8); (5) exercise control rats euthanized 4 h after exercise (n = 8); and (6) exercise rats + DEM euthanized 4 h after exercise (n = 6). DEM rats were given an intraperitoneal injection of 3 mmol/kg body mass DEM dissolved in extra light olive oil and control animals were injected with the extra light olive oil 2 h prior to being sacrificed or exercised. Exercising animals ran on the treadmill at a 10% gradient at 20 m/min for the first 30 min. The speed was then increased every 10 min by 1.6 m/min until exhaustion. The red gastrocnemius and blood was taken under a surgical plane of anaesthesia. Markers of mitochondrial biogenesis such as peroxisome proliferator-activated receptor γ, coactivator-1α (PGC-1α) and nuclear respiratory factor-2 (NRF-2) were measured by real-time PCR. Western blots were used to measure the upstream signaling kinases p38 mitogen-activated protein kinase (p38 MAPK) cAMP-response element binding protein(CREB), and endogenous antioxidants enzymes glutathione peroxidase-1 (GPx-1), superoxide dismutase 2 (SOD2) were measured. All data were tested for normality and a two-way ANOVA was performed. Significance was assumed when $P < 0.05$.

Directly after exercise, there was an overall reduction in total glutathione in the DEM administered animals compared to the control animals, with no differences between the DEM sedentary and exercise group ($p < 0.05$ interaction between exercise and DEM). However, within the control group, total glutathione was higher in the sedentary group compared to animals killed directly after exercise. In addition, DEM significantly increased oxidative stress directly after exercise, as measured by changes in plasma isoprostanes concentration ($p < 0.05$ main effect for DEM). Four hours after exercise, PGC-1α mRNA was significantly increased in both control and DEM administered animals ($p < 0.05$ interaction between exercise and DEM). Furthermore, exercising animals given DEM showed a significantly greater increase in PGC-1α mRNA compared to the control animals that were exercised. NRF-2 mRNA was not altered by either DEM administration or exercise. Directly after exercise, phosphorylation of p38 MAPK protein was significantly increased within the control and DEM administration groups ($p < 0.05$ main effect for exercise). Phosphorylated CREB protein was not altered as a result of exercise or DEM administration. In the control group, exercise resulted in an increase in GPx mRNA compared to the sedentary animals 4 h after exercise, however, DEM administration did not result in any differences between the sedentary and exercise animals ($p < 0.05$ interaction between exercise and DEM). In animals that received DEM there was a significant reduction in GPx activity levels in the sedentary and exercise groups ($p < 0.05$ main effect for DEM). Four hours after exercise, SOD2 activity was reduced in the control exercise and DEM exercised animals ($p < 0.05$ main effect of exercise). In addition, in the DEM groups SOD2 activity was also reduced ($p < 0.05$ main effect for DEM).

This study provides novel evidence by reducing endogenous antioxidant glutathione, skeletal muscle oxidative stress was increased during exercise, resulting in greater PGC-1α gene expression. Thus highlighting the important role of redox balance in the adaptive processes to endurance exercise.

Gomez-Cabrera MC, Borras C, Pallardo FV, Sastre J, Ji LL & Vina J. (2005) *Journal of Physiology* **567**, 113-20.

Higashida K, Kim SH, Higuchi M, Holloszy JO & Han DH. (2011) *American Journal of Physiology-Endocrinology and Metabolism* **301**, E779-84.

Wadley GD & McConell GK. (2010). *Journal of Applied Physiology* **108**, 1719-26.

Wadley GD, Nicolas MA, Hiam DS & McConell GK. (2013) *American Journal of Physiology- Endocrinology and Metabolism* **304**, E853-62.