

PERSPECTIVES

Antioxidants and exercise: a tale of the complexities of relating signalling processes to physiological function?G. L. Close¹ and M. J. Jackson²¹Research Institute for Sport and Exercise Science, Liverpool John Moores University, Liverpool L3 3AF, UK²MRC-Arthritis Research UK Centre for Integrated Research into Musculoskeletal Ageing (CIMA), Department of Musculoskeletal Biology, Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool L69 3GA, UK

Email: mjj@liv.ac.uk

The article by Paulsen and colleagues in this issue of *The Journal of Physiology* ('Vitamin C and E supplementation hampers cellular adaptation to endurance training in humans: a double-blind randomized controlled trial' Paulsen *et al.* 2014) is a useful addition to the debate that has raged in this area and provides some explanation of how the different parties may have come to their diametrically opposing views. In brief, the current study used a formal double-blind trial approach to address this issue and observed that vitamin E and C supplements blunted some cellular adaptations (training-induced increases in muscle mitochondrial proteins), but had no effect on physiological indicators of performance ($\dot{V}_{O_{2max}}$ etc.).

Researchers have been attempting to suppress the assumed deleterious effects of reactive oxygen and nitrogen species that are generated during exercise since the first descriptions of their generation in such situations (e.g. Dillard *et al.* 1978) with little evidence that such interventions were beneficial. However, the realisation that these species play important roles in cell signalling (redox signalling) prompted a rethink of what the so-called 'antioxidants' were meant to achieve. Our group initially demonstrated that high doses of vitamin C could inhibit rapid stress responses to acute exercise (Khassaf *et al.* 2003) and this line was pursued by others who reported that high doses of antioxidants could reduce the training effects of exercise on muscle mitochondrial

biogenesis, $\dot{V}_{O_{2max}}$ and improvements in insulin sensitivity (e.g. Gomez-Cabrera *et al.* 2008; Ristow *et al.* 2009). Although not overtly stated, such studies imply that reactive oxygen and nitrogen species must play a key and possibly unique role in regulating multiple training-induced adaptations to muscle in humans and animals. Unfortunately such findings could not be repeated by other eminent scientists who firmly reported normal adaptations to exercise training despite administration of high dose antioxidants (e.g. Higashida *et al.* 2011). This difference resulted in an intense head-to-head debate in the scientific literature from the groups reporting these differing results (e.g. Holloszy *et al.* 2012).

So why do these studies (and their proponents) differ? It is easy to point out the multiple differences in experimental design that are likely to underlie the variability in reported outcome. These include: the use of animals or humans; trained or untrained subjects; the durations and protocols for the training; the choice of markers of oxidative stress (including some use of completely outdated assays, such as TBARS); the time points studied; the use of muscle *versus* blood markers; and many more potential factors. Consequently, we should not be surprised at the heterogeneity of the reported effects. It is also relevant to question the rationale behind using the particular antioxidant supplements given in these training experiments. Thus why did some investigators give vitamin E supplements for a period of time that does not allow sufficient time for tissue accretion, or why were excessively high vitamin C supplements used in both human and animal models despite widely acknowledged data describing vitamin C saturation kinetics in man and animals and the potential for non-redox effects at these high doses? It is also relevant to consider the current knowledge of redox signalling in muscle and other tissues, and what is clear from the basic biological research undertaken in this area is that these effects are highly site-specific and relatively subtle, such that reactive oxygen and nitrogen species are unlikely to represent a unique regulatory factor in multiple pathways associated with responses to exercise.

The article by Paulsen *et al.* does begin to illustrate this and the complexity of relating signalling processes to true physiological function. The double-blind, randomised design they used gives confidence in the validity of the physiological measurements and appears to confirm that these supplements do not universally inhibit major physiological adaptations to exercise training, although they inhibit potentially relevant changes in mitochondrial proteins. The rationale for otherwise healthy individuals taking such large dose antioxidant supplements in any situation is not clear and an unbiased observer might question why this is an important area. Unfortunately the widespread use of supplements within the sport communities means that this is more than just an interesting scientific question. Facetiously we might propose that if reactive oxygen and nitrogen species were the key regulators of all training-induced adaptations to muscle, we should consider the consumption of *pro-oxidants* (such as peroxides?) to enhance training responses, but the danger is that in the sports arena this might well be attempted!

Our real conclusion from reading this new paper is that we should continue to eat our fruit and vegetables to obtain the necessary antioxidants, as well as to train smart, and we will continue to reap the health benefits.

References

- Dillard CJ, Litov RE, Savin WM, Dumelin EE & Tappel AL (1978). Effects of exercise, vitamin E, and ozone on pulmonary function and lipid peroxidation. *J Appl Physiol Respir Environ Exerc Physiol* **45**, 927–932.
- Gomez-Cabrera MC, Domenech E, Romagnoli M, Arduini A, Borrás C, Pallardo FV, Sastre J & Viña J (2008). Oral administration of vitamin C decreases muscle mitochondrial biogenesis and hampers training-induced adaptations in endurance performance. *Am J Clin Nutr* **87**, 142–149.
- Higashida K, Kim SH, Higuchi M, Holloszy JO & Han DH (2011). Normal adaptations to exercise despite protection against oxidative stress. *Am J Physiol Endocrinol Metab* **301**, E779–E784.
- Holloszy JO, Higashida K, Kim SH, Higuchi M & Han DH (2012). Response to letter to the editor by Gomez-Cabrera *et al.* *Am J Physiol Endocrinol Metab* **302**, E478–E479.

- Khassaf M, McArdle A, Esanu C, Vasilaki A, McArdle F, Griffiths RD, Brodie DA & Jackson MJ (2003). Effect of vitamin C supplements on antioxidant defence and stress proteins in human lymphocytes and skeletal muscle. *J Physiol* **549**, 645–652.
- Paulsen G, Cumming KT, Holden G, Hallén J, Rønnestad BR, Sveen O, Skaug A, Paur I, Bastani NE, Østgaard HN, Buer C, Midttun M, Freuchen F, Wiig H, Ulseth ET, Garthe I, Blomhoff R, Benestad HB & Raastad T (2014). Vitamin C and E supplementation hampers cellular adaptation to endurance training in humans: a double-blind randomized controlled trial. *J Physiol* **592**, 1887–1901.
- Ristow M, Zarse K, Oberbach A, Klötting N, Birringer M, Kiehntopf M, Stumvoll M, Kahn CR & Blüher M (2009). Antioxidants prevent health-promoting effects of physical exercise in humans. *Proc Natl Acad Sci U S A* **106**, 8665–8670.