Response to letter to the editor by Gomez-Cabrera et al.

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TO THE EDITOR: In their Letter to the Editor regarding our paper [Higashida K, Kim SH, Higuchi M, Holloszy JO, Han D-H. Normal adaptations to exercise despite protection against oxidative stress (4)], Gomez-Cabrera et al. (3a) say, “Higashida et al. compared their data with ours and came to exactly the opposite conclusion, i.e., that antioxidant vitamin supplementation does not have an inhibitory effect on the adaptive responses of skeletal muscle to exercise.” In our study, we used standard methodology with which we have years of experience to evaluate the claim by Gomez-Cabrera et al. (3) and Ristow et al. (10) that antioxidant vitamins prevent the adaptive responses to endurance exercise. Our results obtained on rats (4) and those of Bente Pedersen’s group (14, 15) on humans show that supplementation with vitamins C and E has no effect on the adaptive responses of insulin action or of muscle mitochondria to training. The purpose of the letter by Gomez-Cabrera et al. is not clear. Their only comments relating to our paper are: “Higashida et al. (4) misquoted a few times the results obtained in our human study” and “Higashida et al. did not run any performance tests . . . .” We cannot respond to the first comment, as they did not make clear in what way we misquoted their paper. It is true that we did not run any performance tests. Gomez-Cabrera et al. (3) attributed the reduction in endurance to prevention of the training-induced increase in muscle mitochondria by antioxidant vitamins. Because our results show that vitamins C and E have no effect on the adaptive increase in mitochondria, we saw no reason to do performance tests. However, in a study on humans, Yfantl et al. (14) showed that antioxidant vitamins do not prevent training-induced improvements in exercise performance, as have a number of previous studies (6, 11, 12; see also Ref. 2). Gomez-Cabrera et al. make no attempt in their letter to respond to or refute our comments regarding the design of their studies or their data in the discussion section of our paper. The reasons for our opinion that their data provide no support for their claim that antioxidant vitamins prevent the adaptive responses to endurance exercise-training are detailed below.

In the study by Gomez-Cabrera et al. (3), the authors concluded that vitamin C decreases exercise-induced mitochondrial biogenesis in skeletal muscle. This conclusion was based on the claim that vitamin C prevents upregulation of the “PGC-1-NRF-1-mTFA-cytochrome c pathway” in response to exercise training. Gomez-Cabrera et al. state: “The . . . animals trained for 3 wk had significantly higher . . . muscle protein concentrations of PGC-1 . . . which was followed by subsequent increases in the mRNA concentrations of NRF-1 and mTFA. The changes in mRNA were followed by changes in the protein concentrations of . . . these factors . . . in . . . rats trained for 6 wk.” This is an amazing claim, because the PGC-1 protein and NRF-1 and mTFA mRNA assays were all measured in the same six muscles taken 48 h after the last exercise. To obtain information regarding the time course of these events it would be necessary to take muscles at different time points after exercise. Furthermore, increases in PGC-1α protein and NRF-1 and mTFA mRNAs are not markers of training but occur acutely in response to single bouts of exercise (8, 9, 13). The only measurement made by Gomez-Cabrera et al. (3) that could have provided information regarding a training-induced increase in mitochondria is cytochrome c. However, although the authors claimed that vitamin C prevented an exercise-induced increase in cytochrome c, the approximately twofold increase in cytochrome c in the vitamin C-treated trained group shown in Fig. 4 of Gomez-Cabrera et al. (3) does not appear to be significantly different from the value for their control trained group. Furthermore, the authors measured “cytochrome c in the cytosolic fraction,” which has no obvious relevance to cytochrome c in the mitochondrial fraction.

In the study by Ristow et al. (10), previously trained and untrained groups of men underwent 4 wk of training with or without supplementation with vitamins C and E. Ristow et al. reported that the training resulted in large increases in insulin-mediated glucose disposal (euglycemic clamp) and in muscle mRNA levels of PGC-1α, PGC-1β, PPARγ, and superoxide dismutase (SOD)1 and -2 and that the antioxidant vitamins prevented these adaptations. The findings reported by Ristow et al. are puzzling, because the euglycemic-hyperinsulinemic clamps and muscle biopsies were done 7 days after training was stopped. The improvement in insulin-stimulated glucose disposal that occurs in response to training reverses rapidly after cessation of training and disappears within 3–4 days and is no longer present after 7 days (1, 5, 7). The finding of a persistent, highly significant increase in “insulin sensitivity” in the no suppl. trained group (but not in the suppl. trained group) reported in their Fig. 1 by Ristow et al. is, therefore, extremely puzzling. In a study done to evaluate the claims by Ristow et al. (10), Bente Pedersen’s group found identical improvements in insulin-stimulated glucose uptake with training in their antioxidant and placebo groups (15). Ristow et al. measured mRNA levels of PGC-1α, PPARγ, and SOD1 and SOD2 in muscle biopsies taken 7 days after the last training sessions. The increases in mRNAs induced by exercise occur in response to single bouts of exercise and occur regardless of whether or not an individual is trained or not and therefore are not markers for the trained state (8, 9, 13). The exercise-induced increases in mRNAs are transient and reverse rapidly. For example, Perry et al. (8) and Pilegaard et al. (9) have shown, in studies on young men, that PGC-1α mRNA was increased 2-h after each of a series of exercise sessions and had returned to baseline (i.e., preexercise) level 24 h after each exercise bout. Therefore, the finding reported by Ristow et al. that PGC-1α, PPARγ, and PGC-1β mRNAs were still increased approxi-
mately threefold in muscle 7 days after the last training session in the no suppl. (but not in the suppl.) group (their Fig. 2) seems difficult to explain or accept. In contrast, in our study on rats, we measured five mitochondrial proteins and PGC-1α, SOD2, and GLUT4 proteins in muscle taken the day after the last bout of exercise and found similar two- to threefold increases in the vitamins C and E-supplemented and control groups (4).

In light of the results of our study (4), the studies of Yfanti and colleagues (14, 15) and earlier studies (6, 11, 12), we conclude that there is no evidence that antioxidant vitamins interfere with the adaptive responses to endurance exercise training.

REFERENCES