British Journal of Nutrition (2006), **96**, Suppl. 1, S34–S37
DOI: 10.1079/BJN20061697
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https://doi.org/10.1079/BJN20061697 Published online by Cambridge University Press

Relationship of vitamin E metabolism and oxidation in exercising human subjects

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During endurance exercise, oxygen consumption by the skeletal muscle can increase 100–200 times. We previously found that during an ultramarathon race (50 km, forest trail through hilly terrain) compared with a day of rest, vitamin E disappeared faster (as measured using ^{2}H-labelled α-tocopherol) and lipid peroxidation increased. Therefore, we hypothesized that prior supplementation with antioxidants (vitamins E and C) would decrease oxidative stress during distance running and, therefore, decrease lipid peroxidation and inflammation, decrease DNA damage, decrease muscle damage and/or improve recovery. To test these hypotheses, we carried out a randomized, double-blind study in runners (*n* 11 females, 11 males) who were participants in an annual ultramarathon race. We found that supplementation with both vitamins E and C only prevented increases in lipid peroxidation, but had no apparent effect on DNA damage, inflammation or muscle damage. These results suggest that the mechanism of oxidative damage is operating independently of the inflammatory and muscle damage responses.

Ultramarathon: α-Tocopherol: Cytokines: Inflammation: Muscle damage: F_2-isoprostanes

The body continuously produces reactive oxygen species (ROS) as a result of normal oxidative metabolism in the mitochondria. Electrons 'leaking' from the mitochondria during exercise are considered a main source of oxidative stress (Halliwell & Gutteridge, 1999). During endurance exercise, oxygen consumption by the skeletal muscle can increase 100–200 times (Halliwell & Gutteridge, 1999). Thus, exercise causes oxidative stress resulting in lipid peroxidation (Duthie *et al.* 1990; Rokitski *et al.* 1994; Marzatico *et al.* 1997; Child *et al.* 1998; Alessio, 2000; Hessel *et al.* 2000; Mastaloudis *et al.* 2001). Exercise-induced ROS have been suggested to modulate acute phase inflammatory responses (Cannon & Blumberg, 2000).

We found that during an ultramarathon race (50 km, forest trail through hilly terrain) compared with a day of rest, vitamin E disappeared faster (as measured using ^{2}H-labelled α-tocopherol) and lipid peroxidation increased (Mastaloudis *et al.* 2001). Lipid peroxidation was assessed by measuring plasma F_2-isoprostanes (F_2-IsoP).

F_2-IsoP are prostaglandin-like compounds produced by the free-radical catalysed oxidation of arachidonic acid (20 : 4 *n*-6, a long-chain PUFA; Morrow *et al.* 1990) and are widely accepted as a sensitive and reliable measure of *in vivo* lipid peroxidation (Roberts 1997). Importantly, F_2-IsoP have pro-atherogenic biological activity, including vasoconstriction and activation of platelet aggregation (Roberts & Morrow, 2000); they recruit monocytes and induce their adhesion (Roberts & Morrow, 2000). Moreover, a reduction in cardiovascular risk is associated with a decrease in isoprostanes (Morrow, 2005). Thus, endurance exercise not only increases oxidative stress, it increases a pro-atherogenic response.

Modulation of oxidative stress during an ultramarathon by vitamins E and C

Based on our findings that the ultramarathon race increased oxidative stress (Mastaloudis *et al.* 2001), we hypothesized that prior supplementation with antioxidants (vitamins E and C) would decrease oxidative stress during distance running. If the antioxidant supplements decrease oxidative stress, they should, therefore, decrease lipid peroxidation and inflammation (Mastaloudis *et al.* 2004*a*), decrease DNA damage (Mastaloudis *et al.* 2004*b*), decrease muscle damage and/or improve recovery (Mastaloudis *et al.* 2006). To test these hypotheses, we carried out a randomized, double-blind study in runners (*n* 11 females, 11 males) who were participants in an annual race, a 50 km (31 mile) ultramarathon that takes place in the hills of Corvallis, Oregon. Subjects were randomly assigned to consume either: (1) antioxidants (300 mg vitamin E, 400 IU *RRR*-α-tocopheryl acetate) and 1000 mg vitamin C (500 mg twice/d) or (2) matching placebos for 6 weeks pre-race, 1 week post-race. Blood samples were obtained prior to supplementation (baseline), following 3 weeks supplementation (compliance), 24, 12, 1 h prior to the race (pre-race, 0 h), in the middle of the race at kilometre 27 (mid-race, ~5 h), immediately post-race, 2 h after race end (2 h post-race, ~10 h) and daily for 6 d following the race (post 1–6 d, 24–144 h). All samples were fasting morning blood draws except −12 h, mid-, post- and 2 h post-race.

Subject characteristics and plasma antioxidant concentrations

Subjects were approximately 40 years old and were recreationally trained endurance runners. Complete descriptions of

Abbreviation: F_2-IsoP, F_2-isoprostanes; ROS, reactive oxygen species.

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https://doi.org/10.1079/BJN20061697 Published online by Cambridge University Press

the subjects have been published (Mastaloudis *et al.* 2004*a*,*b*).

At baseline (prior to supplementation), plasma α-tocopherol and ascorbic acid concentrations were not different between the two groups (Mastaloudis *et al.* 2004*b*). Following 6 weeks of supplementation, plasma concentrations were unchanged in the placebo group, while in the antioxidant takers, plasma α-tocopherol concentrations increased from 28 (SD 2) to 45 (SD 3) μM ($P<0{\cdot}0001$) and were higher than in the placebo group ($P<0{\cdot}0007$). Similarly, the antioxidant group following supplementation had higher plasma ascorbic acid concentrations, 121 (SD 9) μM, than did the placebo group, 78 (SD 9) μM ($P<0{\cdot}0007$). It should be noted that the ascorbic acid concentrations in both groups were well within concentrations that should saturate tissues with ascorbic acid (Levine *et al.* 1996, 2001).

All subjects completed the race. Run times and intensity were similar between treatment groups and genders: 7·1 (SD 0·2) h at a pace of 13·7 (SD 0·4) min/mile and heart rate of 146 (SD 2) bpm. Energy expenditure was calculated based on average heart rate during the run and the corresponding oxygen consumption (VO_2) multiplied by the time it took each subject to finish the race (Mastaloudis *et al.* 2004*b*). Energy expenditure was approximately 29·3 MJ (7000 kcal) for males and 20·9 MJ (5000 kcal) for females; both genders consumed about 8·3 MJ (2000 kcal) on race day and therefore were in energy deficit. Vitamin E intake during the run was <5 mg, while vitamin C intake was <50 mg for most subjects.

Runners taking vitamins E and C are protected from lipid peroxidation

Similarly to our previous findings (Mastaloudis *et al.* 2001), F_2-IsoP concentrations increased during and at race end in placebo takers, in both men and women. Surprisingly, the responses of men and women during the post-race period were markedly different. In placebo women, F_2-IsoP concentrations returned to baseline concentrations within 2 h post-race, while in placebo men F_2-IsoP concentrations remained elevated for the entire week after the race (Mastaloudis *et al.* 2004*a*). Previously, men have been observed to experience greater oxidative stress than do women (Jenkins & Goldfarb, 1993). Thus, men compared with women are subjected to continued higher oxidative stress especially in the days after the endurance event.

F_2-IsoP concentrations increased in the placebo group during the race, but prior supplementation with vitamins E and C completely abrogated this increase in the antioxidant group (Mastaloudis *et al.* 2004*a*). At post-race, when oxidative stress was maximal, F_2-IsoP concentrations were inversely correlated both with α-tocopherol/lipids ($R=-0{\cdot}61$, $P<0{\cdot}003$) and ascorbic acid ($R=-0{\cdot}41$, $P=0{\cdot}05$) (Mastaloudis *et al.* 2004*a*), providing further documentation that antioxidants were responsible for preventing lipid peroxidation.

Endogenous mechanisms to increase antioxidant defences

In contrast to our finding that F_2-IsoP increased during the race, we also found that plasma ascorbic acid concentrations increased in both the antioxidant and placebo groups during the 50 km ultramarathon run, with significant increases compared with pre-race at mid-race and post-race, returning to pre-race values by 2 h post-race (Mastaloudis *et al.* 2004*a*). Increases in plasma ascorbic acid in response to vigorous exercise have been reported in some (Duthie *et al.* 1990; Viguie *et al.* 1993; Rokitski *et al.* 1994; Kaikkonen *et al.* 1998; Mastaloudis *et al.* 2001; Petersen *et al.* 2001), but not all (Meydani *et al.* 1993; Peters *et al.* 2001*a*,*b*) exercise studies. Exercise-related increases in circulating cortisol have been suggested to promote efflux of ascorbic acid from the adrenal gland and/or the mobilization of ascorbic acid from leucocytes or erythrocytes (Gleeson *et al.* 1987). Some (Nieman *et al.* 2000; Peters *et al.* 2001*a*,*b*), but not all (Nieman *et al.* 2002), studies have demonstrated an attenuation of the exercise-related increase in circulating cortisol with vitamin C supplementation. Taken together, these findings suggest that oxidative stress and ascorbic acid may coordinately regulate cortisol secretion.

Plasma uric acid concentrations also increased in response to the run, consistent with the findings of others (Rokitski *et al.* 1994; Hellsten *et al.* 1997; Liu *et al.* 1999). The increase may be explained by enhanced purine oxidation with exercise (Rokitski *et al.* 1994; Hellsten *et al.* 1997; Liu *et al.* 1999). Concurrent increases in plasma ascorbic and uric acids may reflect the body's response to extreme exercise by enhancing antioxidant defences including increased antioxidant enzymes (Marzatico *et al.* 1997) and antioxidant nutrients (Viguie *et al.* 1993; Rokitski *et al.* 1994; Child *et al.* 1998).

A few of the studies investigating the effects of vitamin E supplementation on endurance running have reported increases in plasma α-tocopherol concentrations in both supplemented and placebo groups following exercise (Rokitski *et al.* 1994; Vasankari *et al.* 1997; Buchman *et al.* 1999). We observed an increase in plasma α-tocopherol concentrations during exercise in the antioxidant, but not the placebo group. Plasma tocopherols are transported entirely within lipoproteins and fluctuate with lipoprotein concentrations (Traber & Jialal, 2000). After correcting α-tocopherol for lipids, no significant changes in α-tocopherol/lipids were observed in either group. In the only other study to report both α-tocopherol and α-tocopherol/lipid concentrations (Buchman *et al.* 1999), differential responses in the antioxidant- and placebo-supplemented groups were also explained by fluctuations in lipoproteins. Thus, the increase in plasma α-tocopherol concentrations during the exercise may be a result of fluctuations in plasma lipoprotein concentrations.

Increased cytokines in response to the race

In contrast to their effects on lipid peroxidation, we found that antioxidant supplementation had no effect on exercise-induced increases in cytokines, such as TNF-α, IL-6, C-reactive protein, IL-1 or ferritin (Mastaloudis *et al.* 2004*a*). Short-term (1 week) ascorbic acid (1500 mg/d) supplementation prior to an ultramarathon did not prevent exercise-induced increases in plasma F_2-IsoP, lipid hydroperoxides or IL-6 (a pro-inflammatory cytokine; Nieman *et al.* 2002). Similarly, 1 week post-exercise supplementation with vitamin C and *N*-acetyl-cysteine had no effect on exercise-induced IL-6 increases (Childs *et al.* 2001). Additionally, C-reactive protein was not attenuated by antioxidant supplementation (Brull *et al.* 2004). In general, antioxidants do not appear to modulate cytokine concentration increases in response to exercise.

https://doi.org/10.1079/BJN20061697 Published online by Cambridge University Press

Assessment of DNA damage using the comet assay

The ultramarathon run was sufficiently strenuous that by mid-race, subjects exhibited DNA damage, as assessed using the comet assay (Mastaloudis *et al.* 2004*b*). Nonetheless, the proportion of cells with DNA damage returned to baseline by the end of the race, by 2 d after the race the values declined below baseline values, and remained depressed at 6 d post-race (Mastaloudis *et al.* 2004*b*). Both men and women within each treatment group had similar circulating antioxidant levels, but women runners had higher levels of DNA damage. Moreover, antioxidant supplementation protected the women runners by decreasing the proportion of cells with damage on the day following the ultramarathon race, while men experienced little benefit from the antioxidants.

Overall, exercise appears to induce a temporary increase in DNA damage. This increase, however, does not appear to have adverse effects, and may be beneficial because it appears to induce removal of damaged cells.

Muscle damage following running

Following the ultramarathon race, runners experienced muscle damage. Deficits in maximal force production of the knee extensors and flexors have been documented (Mastaloudis *et al.* 2006). Prior supplementation with vitamins E and C did not alleviate muscle damage or fatigue nor improve recovery. Possibly, the ultramarathon run was so damaging that it overwhelmed the protective effects of the antioxidants. Previously, vitamin C was found to be protective in a more moderate exercise protocol, 60 min of box-stepping exercise, where supplementation enhanced the rate of recovery of maximal force deficit (Jakeman & Maxwell, 1993).

Plasma markers of muscle damage were increased by the endurance exercise and unaffected by antioxidant supplementation (Mastaloudis *et al.* 2005). When exercisers underwent 2 week supplementation with 400 mg vitamin E and 500 mg vitamin C prior to a 90 min treadmill run, exercise-related increases in creatine kinase still occurred (Petersen *et al.* 2001). However, 4·5 week supplementation with 400 IU vitamin E (400 mg DL-α-tocopherol) and 200 mg vitamin C attenuated increases in creatine kinase following a 90 km ultramarathon (Rokitski *et al.* 1994).

Our study also showed that vitamins E and C did not prevent increases in lactic dehydrogenase following distance running. However, Itoh *et al.* (2000) reported that supplementation with 1200 IU α-tocopherol (400 mg DL-α-tocopheryl acetate) daily for 4 weeks attenuated lactic dehydrogenase increases following six successive days of running, suggesting that a larger dose of vitamin E is required to attenuate muscle damage resulting from endurance exercise. Thus, outcomes appear to be influenced not only by level of exercise, but also by the amount, duration and the type of supplement antioxidant.

Conclusions

In response to endurance exercise, ROS are generated causing oxidative damage (Mastaloudis *et al.* 2001), inflammatory responses are stimulated (Vassilakopoulos *et al.* 2003) and skeletal muscle is damaged (Sjodin *et al.* 1990; Cannon & Blumberg, 2000). Hypothetically, antioxidant supplementation could prevent exercise-induced oxidative damage, inflammation and muscle damage. However, we found that supplementation with both vitamins E and C only prevented increases in lipid peroxidation (Mastaloudis *et al.* 2004*a*), but had no apparent effect on DNA damage (Mastaloudis *et al.* 2004*b*), inflammation (Mastaloudis *et al.* 2004*a*), or muscle damage (Mastaloudis *et al.* 2006). These results suggest that the mechanism of oxidative damage is operating independently of the inflammatory and muscle damage responses (Nieman *et al.* 2002). Both vitamins C and E are likely to be needed in exercise. The ultramarathon runners in the present study were adequately nourished with respect to vitamin C, but studies in cigarette smokers suggest that vitamin C is necessary to prevent enhanced vitamin E disappearance rates (Bruno *et al.* 2005).

Preventing production and or enhancing clearance of F_2-IsoP may be more beneficial than preventing inflammation because F_2-IsoP have demonstrated pro-atherogenic biological activity. In contrast, the muscle damage-induced inflammatory response stimulates recovery from exercise by inducing regeneration of damaged tissue and recruitment of satellite cell proliferation (Malm, 2001). Together, antioxidant supplementation proved to prevent the damaging increase in lipid peroxidation without influencing inflammation. This is especially important since prevention of exercise-induced inflammation could inhibit muscular adaptation to physical activity, the so-called 'training effect' of exercise.

Acknowledgements

The runners! Supported by NIEHS grants ES11536 and NIH DK59576. The following individuals provided supplements and ergogenic aids: Jim Clark (Cognis Health and Nutrition); Klaus Krämer (BASF); Tim Corliss (Clif Bar); Jeff Zachwieja (Gatorade). The following individuals played key roles in our study: Angela Mastaloudis and Dawn W. Hopkins (Department of Exercise and Sport Science, and Linus Pauling Institute, Oregon State University (OSU), Corvallis, OR); Scott Leonard, David Yu, Roderick H. Dashwood and Balz Frei (Linus Pauling Institute, OSU); Robert P. O'Donnell (Department of Statistics, OSU); Jason D. Morrow (Vanderbilt University School of Medicine, Nashville, TN); Sridevi Devaraj (University of California Medical Center, Sacramento, CA).

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