

## ***Neutrophil activation, antioxidant supplements and exercise-induced oxidative stress***

**Running Title:** Neutrophils, oxidative stress and antioxidants

**Jonathan Peake and Katsuhiko Suzuki**

**School of Human Sciences and Consolidated Research Institute for Advanced Science and Medical Care, Waseda University, 2-579-15,**

### **Abstract**

*Neutrophils produce free radicals known as reactive oxygen species (ROS), which assist in the clearance of damaged host tissue. Tissue damage may occur during exercise due to muscle damage, thermal stress and ischaemia/reperfusion. When produced in excess, neutrophil-derived ROS may overwhelm the body's endogenous antioxidant defence mechanisms, and this can lead to oxidative stress. There is increasing evidence for links between oxidative stress and a variety of pathological disorders such as cardiovascular diseases, cancer, chronic inflammatory diseases and post-ischaemic organ injury. A small number of studies have investigated whether there is a link between neutrophil activation and oxidative stress during exercise. In this review, we have summarised the findings of these studies. Exercise promotes the release of neutrophils into the circulation, and some evidence suggests that neutrophils mobilised after exercise have an enhanced capacity to generate some forms of ROS when stimulated in vitro. Neutrophil activation during exercise may challenge endogenous antioxidant defence mechanisms, but does not appear to increase lipid markers of oxidative stress to any significant degree, at least in the circulation. Antioxidant supplements such as N-acetylcysteine are effective at attenuating increases in the capacity of neutrophils to generate ROS when stimulated in vitro, whereas vitamin E reduces tissue infiltration of neutrophils during exercise. Free radicals generated during intense exercise may lead to DNA damage in leukocytes, but it is unknown if this damage is the result of neutrophil activation. Exercise enhances the expression of inducible haem (heme)-oxygenase (HO-1) in neutrophils after exercise, however, it is uncertain whether oxidative stress is the stimulus for this response.*

**Keywords:** N-acetylcysteine, vitamin C, vitamin E, antioxidant supplements, free radical scavengers.

### **Introduction**

Oxidative stress has been defined as an increase in free radical production that results from tissue damage. There are numerous potential sources of free radical

---

*Address correspondence to:*

Mikajima, Tokorozawa, Saitama 359-1192, Japan.

Tel & Fax: +81 4-2947-6898, Email: katsu.suzu@waseda.jp

production within the body, and one of these sources is activated neutrophils (11). Neutrophils produce free radicals known as reactive oxygen species (ROS), which form the central component of neutrophil defence mechanisms against foreign pathogens during infection, and damaged host tissue fragments following injury (48). The main ROS generated in the neutrophil respiratory burst are superoxide anion ( $O_2^-$ ). These superoxide anions are then converted to other ROS, including hydrogen peroxide ( $H_2O_2$ ), hypochlorous acid (HOCl), hydroxyl radicals ( $\cdot OH$ ) and singlet oxygen ( $^1O_2$ ) (see Figure 1) (31). While neutrophil-derived ROS play an important role in breaking down damaged fragments of host tissue (48), when produced in excess, these toxic molecules may also contribute to oxidative stress. This involvement of neutrophils could have important implications for the development of a variety of pathological conditions that have been linked with oxidative stress, including atherosclerosis, cancer, rheumatoid arthritis and post-ischaemic organ injury (11).

Some forms of exercise increase the capacity of neutrophils to generate ROS when stimulated *in vitro*, possibly in response to exercise-induced muscle damage (33, 45). There is also evidence that exercise may promote oxidative stress (20, 24, 41). A small number of studies have examined the link between neutrophils and oxidative stress after exercise. The aim of this brief review is to summarise the findings from these studies, and to assess the evidence as to whether increased neutrophil activation after exercise is related to oxidative stress.

### Tissue injury, ROS and oxidative stress during exercise

Tissue injury is the most common cause of oxidative stress. During exercise, tissue injury may result from muscle damage, thermal stress and ischaemia/reperfusion. Tissue injury is characterised by physiological alterations including impaired antioxidant defence systems, release of arachidonic acid, metal ions and haem proteins, and activated neutrophils – all of which are potential sources of free radicals and ROS production (11). When produced in excess, free radicals oxidise lipids, proteins and nucleic acids. These reactions can lead to lipid peroxidation, impaired physiological function and DNA damage (26), and these adverse changes can result in pathological disorders such as those mentioned above (11).

A large number of studies have investigated the effects of exercise on markers of oxidative stress. However, the validity of many of the markers has been questioned because of their lack of specificity. Consequently, it has been difficult to draw definitive conclusions on the issue of whether exercise does cause oxidative stress (17). More recent exercise studies have used plasma  $F_2$ -isoprostanes as an indicator of lipid peroxidation. There are reports of increased plasma  $F_2$ -isoprostane levels after eccentric forearm exercise (6), prolonged running (20, 41) and an ultraendurance triathlon event (24). Therefore, various forms of exercise may increase lipid markers of oxidative stress.

Exercise scientists have proposed a number of sources of ROS production during exercise, including the mitochondrial electron transport chain, the xanthine oxidase system, metal-catalysed reactions and activated neutrophils (26). Several groups have focused more specifically on neutrophils as a source of

ROS production, adopting a variety of different approaches. Firstly, some groups have examined the influence of antioxidants on post-exercise alterations in the capacity of neutrophils to generate ROS upon *in vitro* stimulation (4-6, 15, 18, 22, 23, 38). Secondly, some groups have examined whether alterations in neutrophil activation lead to changes in markers of oxidative stress and endogenous antioxidant defences (14, 34). Lastly, others have investigated the effects of endogenous antioxidants by incubating neutrophils with pre- or post-exercise plasma and measuring their capacity to generate ROS upon *in vitro* stimulation (19, 39, 43).

### Exercise-induced neutrophil activation

Neutrophils may be activated during exercise by a variety of factors including muscle damage, growth hormone and interleukin (IL)-6 (31, 45). Reports of alterations in the capacity of neutrophils to generate ROS upon *in vitro* stimulation following exercise are variable. After brief, maximal exercise, some groups have reported an increase, whereas others have reported a decrease. The responses to strenuous prolonged exercise are similarly inconsistent (for review see 31, 44). This disparity may be due to a number of factors including differences in sampling times, the assay techniques used to measure *in vitro* neutrophil ROS production, exercise mode, and the fitness levels of participants.

Intense and/or prolonged exercise elicits a large increase in the number of circulating neutrophils, and there is evidence to suggest that these newly recruited neutrophils have an increased capacity to generate ROS. Our laboratory has performed several studies investigating changes in the capacity of neutrophils to generate ROS upon *in vitro* stimulation after brief maximal exercise (44), and also after prolonged cycling at moderate intensity (45) and higher (42). The results of each of these studies indicated a significant increase in the proportion of either segmented (mature) or band (immature) neutrophils after exercise, depending on the duration or intensity, respectively. These increases were accompanied by enhanced production of HOCl, as measured by luminol-enhanced chemiluminescence. In two of the studies above (42, 44), *in vitro* neutrophil ROS production was positively correlated with the number of segmented neutrophils. There was a minor nonsignificant decline in the production of  $O_2^-$  after exercise, as measured by lucigenin-enhanced chemiluminescence (44). These data suggest that brief and prolonged exhaustive exercise promotes the mobilisation of a subset of neutrophils (segmented) with enhanced capacity to metabolise ROS such that the production of some types of neutrophil-derived ROS (e.g. HOCl) increases. In contrast, other types of substrate-level ROS (e.g.  $O_2^-$  and  $H_2O_2$ ) may be consumed or neutralised during exercise, possibly through the action of myeloperoxidase (44, see Figure 1).

### The effects of antioxidant supplementation on *in vitro* neutrophil ROS production

*N*-acetylcysteine and vitamin C are the most commonly used antioxidants in studies investigating the influence of antioxidant supplementation on post-exercise alterations in the capacity of neutrophils to generate ROS upon *in vitro* stimula-

tion. *N*-acetylcysteine is a water-soluble precursor of glutathione. Glutathione is an endogenous antioxidative thiol that reacts with ROS either directly, or indirectly as a cofactor of the antioxidant enzyme glutathione peroxidase (15, 37). A number of *in vitro* trials have shown that *N*-acetylcysteine enhances glutathione synthesis and modulates leukocyte activity (29, 40, 47). Vitamin C is also a water-soluble antioxidant that is stored in high concentrations within neutrophils to regulate the production of ROS (16). There is evidence from *in vitro* studies indicating that vitamin C has a strong regulatory influence on neutrophil production of ROS (3, 8). Vitamin C appears to act directly by scavenging HOCl (2).

Huupponen *et al.* supplemented untrained males with 200 mg *N*-acetylcysteine per day for two days prior to incremental cycling to exhaustion (15). *N*-acetylcysteine prevented any increase in the capacity of neutrophils to generate ROS upon *in vitro* stimulation (see Table 1). In another study, a group of rowers received 6 g *N*-acetylcysteine for three days before completing 6 min of maximal exercise on a rowing ergometer (22). In this latter study, *N*-acetylcysteine significantly reduced ( $P < 0.05$ ) *in vitro* neutrophil ROS production below pre-exercise values (see Table 1).

Nieman *et al.* supplemented runners with 1000 mg vitamin C per day for eight days prior to a 2.5-h run at 75-80%  $VO_{2max}$  (23). There appeared to be a small non-significant trend towards reduced *in vitro* neutrophil ROS production below pre-exercise values in the hours after exercise (see Table 1). However, it is difficult to interpret the significance of this result for several reasons. Firstly, there were no significant alterations in the capacity of neutrophils to generate ROS upon *in vitro* stimulation after the placebo trial, which suggests that the exercise protocol itself did not increase *in vitro* neutrophil ROS production. Secondly, the athletes in this study consumed carbohydrate during exercise, which could have influenced the results. Lastly, this study did not involve a cross-over experimental design.

Krause *et al.* reported similar results after they provided a small group of athletes with 2 g vitamin C for one week before intense running and cycling activity (18) (see Table 1). This study was weakened by the small sample size in the supplement ( $n=6$ ) and placebo groups ( $n=4$ ), and the failure to use a cross-over experimental design.

Childs *et al.* supplemented a group of untrained males with 12.5 mg vitamin C and 10 mg *N*-acetylcysteine for seven days after acute muscle injury induced by eccentric exercise (6). Relative to the placebo condition, supplementation significantly increased the plasma concentration of lipid hydroperoxide two and three days after exercise, while there was also a trend ( $P = 0.07$ ) toward elevated plasma  $F_2$ -isoprostane levels. Interestingly, supplementation significantly attenuated ( $-30\%$ ,  $P < 0.05$ ) increases in plasma myeloperoxidase concentration up to seven days post-exercise. Therefore, it was uncertain if neutrophil activation contributed to the increase in oxidative stress seen after exercise. Considering that the exercise used in this study did not involve large aerobic demand, and also that the supplement was given to participants after exercise, it is possible that ischaemia-reperfusion related to muscle damage may have been a greater source of ROS production after exercise. Neutrophil degranulation is linked with the production of ROS, and it is therefore possible that the reduced plasma myeloperoxidase concentration observed in this study following supplementation occurred through an alteration in the intracellular redox status of neutrophils.

Vitamin E is another antioxidant that has been used in exercise studies of this type. Part of the rationale for using vitamin E supplements is that vitamin E can modify arachidonic acid metabolism (10), which forms part of the signal transduction pathway in neutrophil respiratory burst activity (31). Cannon *et al.* (5) investigated the effects of 48 days of supplementation with 800 IU vitamin E on neutrophil ROS production *in vitro* in  $<30$ -year-old and  $>55$ -year-old untrained males. All participants ran downhill on a treadmill for three 15-min periods at 75% maximum heart rate. There was a mean increase of 30% in plasma lipid peroxide concentration 24 h after exercise, but this was not statistically significant. There were also no significant changes in the capacity of neutrophils to generate ROS upon *in vitro* stimulation after exercise, except for a significant increase in the  $>55$ -year-old group five and 12 days after exercise. These responses were not altered by vitamin E.

Aoi *et al.* supplemented rats with vitamin E for three weeks before 60 min of intense treadmill running (4). They reported increased thiobarbituric acid-reactive substances (TBARS) within the gastrocnemius muscle after exercise, which the authors interpreted as indicating tissue oxidation. This increase was significantly attenuated ( $-27\%$ ,  $P < 0.05$ ) by vitamin E supplementation. Aoi *et al.* also found that relative to the placebo, vitamin E significantly reduced the post-exercise increase in muscle myeloperoxidase content ( $-30\%$ ,  $P < 0.05$ ), suggesting that vitamin E may reduce the infiltration of neutrophils into the muscle after exercise. However, the capacity of circulating neutrophils to generate ROS upon *in vitro* stimulation remained unchanged after exercise, and was not affected by vitamin E. In explanation of the above findings, it is possible that neutrophils infiltrating muscle tissue during exercise are activated by chemokines produced within muscle. This activation may be attenuated by exogenous antioxidants such as vitamin E, whereas endogenous might be less easily mobilised. In contrast, endogenous antioxidants may be more readily mobilised into the circulation than into muscle tissue during exercise, thereby preventing activation of neutrophils within the circulation.

In contrast to the findings of the studies above, Robson *et al.* (35) reported that a combination of vitamins A, C and E given to athletes for seven days before 2 h running at 65%  $VO_{2max}$  enhanced the capacity of neutrophils to produce ROS *in vitro* after exercise (see Table 1). A 6-week period of baseline supplementation also enhanced *in vitro* neutrophil ROS production at rest. Robson *et al.* proposed that the antioxidant supplement scavenged ROS produced by neutrophils themselves, thereby preventing auto-oxidation and allowing neutrophils to maintain their capacity to generate ROS to occur (3). They also suggested that a combination of antioxidants such as that given to the athletes in their study may have a greater ROS-scavenging effect than single antioxidant vitamins given to athletes in other studies (5, 18, 23).

Zinc is an important co-factor in a range of immune functions, and athletes may be zinc-deficient (32). Singh *et al.* supplemented a group of trained runners with a zinc/copper combination for four days prior to a run to exhaustion at 75%  $VO_{2max}$  (38). The supplement prevented an increase in the capacity of neutrophils to produce ROS *in vitro* immediately after exercise. The effects of zinc on *in vitro* neutrophil ROS production appear to be dependent on the dose of zinc, and the signal transduction pathway that is activated (13). Because Singh *et al.* (38) did

not measure any antioxidants or lipid markers of oxidative stress, the significance of this finding in the context of exercise-induced oxidative stress is unclear.

The results of these studies suggest that the influence of antioxidants on post-exercise changes in the capacity of neutrophils to produce ROS *in vitro* may depend on the type of exercise, and the type of antioxidant supplement. Antioxidants may be most effective in neutralising ROS produced by neutrophils in response to brief maximal exercise. In contrast, the lower intensity of longer duration exercise may not increase *in vitro* neutrophil ROS production to the level required to observe any influence of antioxidant supplements. This concept is supported by the findings of Quindry *et al.* who compared alterations in the capacity of neutrophils to produce ROS *in vitro* and endogenous antioxidants following exercise at several different intensities. Their results demonstrated that neutrophil ROS production *in vitro* only increased significantly in response to maximal exercise, and furthermore, this increase was associated with decreased plasma concentrations of uric acid and ascorbic acid (34).

Because the exercise protocols used in those studies investigating the effects of vitamin E (4, 5) did not stimulate any significant increase in neutrophil ROS production *in vitro*, the efficacy of vitamin E supplementation remains unknown. Alternatively, *N*-acetylcysteine may be more effective than vitamin C in terms of reducing the production of ROS by neutrophils after exercise. The intracellular redox status of neutrophils is likely to be an important determinant of the capacity of neutrophils to generate ROS (7). It is possible that as a precursor of glutathione, *N*-acetylcysteine may have a stronger influence than vitamin C on the intracellular redox status of neutrophils. In turn, this might account for the greater effects of *N*-acetylcysteine after exercise. Lastly, supplements containing vitamins C or E on their own do not appear to alter *in vitro* neutrophil production of ROS (4, 5, 18, 23). When given to athletes in combination, they may actually promote neutrophil activation (35).

### The effects of neutrophil activation on markers of oxidative stress and endogenous antioxidants

Another approach to examining the role of neutrophils in exercise-induced oxidative stress has focused on measuring simultaneous alterations in the capacity of neutrophils to generate ROS *in vitro*, markers of oxidative stress and endogenous antioxidants after exercise. Hessel *et al.* measured changes in these parameters after a marathon race. They reported a large increase in neutrophil ROS production *in vitro*, together with an increase in plasma lipid peroxide concentration and alterations in various endogenous antioxidants indicative of oxidative stress (see Table 1) (14). In comparison to the large increase in neutrophil ROS production *in vitro* after exercise, the changes in the antioxidants markers of oxidative stress were relatively small. Therefore, the data from this study suggest that increases in neutrophil ROS production *in vitro* after prolonged exercise such as the marathon do not markedly increase oxidative stress or place significant demands on endogenous antioxidant defence mechanisms *in vivo*.

Quindry *et al.* also measured alterations in neutrophil ROS production *in vitro*, markers of oxidative stress and endogenous antioxidants after exercise at different intensities ranging from 10% below lactate threshold up to maximal

exercise (34). The results of their study revealed that only maximal exercise significantly increased neutrophil ROS production *in vitro* (see Table 1). This increase was accompanied by a significant decline in the plasma concentrations of ascorbic acid and uric acid. However, there were no significant changes in markers of oxidative stress such as plasma lipid peroxide and malondialdehyde concentrations. The results of this study suggest that increases in neutrophil ROS production *in vitro* after brief maximal exercise may challenge endogenous antioxidant defence mechanisms, but it is unlikely that neutrophils promote lipid peroxidation following such activity, at least in the circulation.

### Effects of incubating neutrophils with plasma

Several studies have investigated the effects of incubating neutrophils with pre- or post-exercise plasma. In one study from our laboratory, we isolated neutrophils under resting conditions from a healthy individual, and incubated them with plasma obtained before and after a marathon race (43). The results indicated that relative to the blank condition (without plasma), the production of ROS when neutrophils were stimulated *in vitro* decreased significantly by 41% when the neutrophils were incubated with pre-exercise plasma. When the neutrophils were incubated with post-exercise plasma, their capacity to generate ROS *in vitro* decreased significantly by 63%. We also found increased plasma concentrations of uric acid, albumin and vitamin C in addition to enhanced superoxide dismutase and catalase activity after exercise. We proposed that the ROS-scavenging properties of these antioxidants may account for the large decline that they demonstrated when neutrophils were incubated with post-exercise plasma (43). Therefore, under certain conditions endogenous antioxidants released after exercise may neutralise ROS produced by neutrophils, thereby reducing the potential for oxidative stress to occur.

Two other studies have made similar measurements. In their study of 1 h cycling at 50%  $\text{VO}_{2\text{max}}$  in untrained individuals, Macha *et al.* reported a minor non-significant decrease (3%) in neutrophil ROS production *in vitro* when pre-exercise neutrophils were incubated with post-exercise plasma (19). Smith *et al.* carried out a similar experiment, but reported no effect of incubating pre-exercise neutrophils with pre- or post-exercise plasma (39).

The disparate results obtained from these three studies could be due to differences in the types of exercise involved, and the fitness level of the participants. The marathon race in our study above (43) likely increased plasma antioxidant activity to a greater extent than the submaximal exercise used in the other two studies (19, 39). Furthermore, the participants in our study (43) were experienced marathon runners, whereas the participants in the other studies (19, 39) were mainly untrained. There is evidence both for and against the concept that training alters antioxidant defence mechanisms and lipid markers of oxidative stress (17, 21). It is possible that trained athletes have greater capacity to mobilize antioxidants to prevent oxidative stress during exercise. Whether training affects the interaction between neutrophils and endogenous antioxidants during exercise remains to be determined.

Table 1. Summary of studies investigating the relationship between post-exercise alterations in the capacity of neutrophils to generate reactive oxygen species in vitro and markers of oxidative stress.

Subjects	Exercise	Antioxidant supplement	% change in neutrophil activity from pre-exercise	% changes in markers of oxidative stress and endogenous antioxidants	Reference
Untrained (n=9)	VO <sub>2max</sub> cycling test	200 mg N-acetylcysteine	16% ↓ (Post), 22% ↑ (Post)*	-	(15)
Rowers (n=19)	6 minutes maximal rowing	6 g N-acetylcysteine	10% ↓ (Post)*, 33% ↓ (2 h Post)*	-	(22)
Runners (n=30)	2.5 h run at 75-80% VO <sub>2max</sub>	Placebo 1 g vitamin C	18% ↑ (Post)*, 10% ↑ (2 h Post) NC (Post), 13% ↓ (1.5 h), 18% ↓ (post), 16% ↓ (6 h Post)	-	(23)
Duathletes (n=10)	16.5 km uphill cycling, 2.5 km uphill running	Placebo 2 g vitamin C	25% ↑ (Post), NC (1.5 h Post), NC (3 h Post), NC (6 h Post)	-	(18)
Mixed endurance (n=12)	2 h run at 65% VO <sub>2max</sub>	Placebo 18 mg β-carotene, 90 mg vitamin E, 900 mg vitamin C	17% ↓ (Post) 6% ↓ (Post)	-	(35)
Runners (n=18)	Marathon race	Placebo	30% ↑ (Post)* 20% ↓ (Post) 142% ↑ (Post)	11% ↑ (Post) in plasma GSH conc.* 16% ↑ (Post) in plasma GSSG conc.* 8% ↑ (Post) in GSH:GSSG ratio* 10% ↓ (Post) in GSH-Px activity* 7% ↓ (Post) in SOD activity* 11% ↑ (Post) in plasma LPO conc.*	(14)
Active (n=9)	Treadmill VO <sub>2max</sub> test	-	90% ↑ (Post)*, 50% ↑ (1 h Post), 125% ↑ (2 h Post)*	26% ↓ (Post)*, 5% ↑ (1 h Post), 14% ↓ (2 h Post) in plasma ascorbic acid concentration 29% ↓ (Post)*, 30% ↑ (1 h Post)*, 30% ↑ (1 h Post)* in plasma uric acid concentration 41% ↑ (Post), 49% ↑ (1 h Post), 35% ↑ (2 h Post) in plasma lipid peroxide concentration 5% ↓ (Post), 5% ↑ (1 h Post), NC (2 h Post) in plasma malondialdehyde concentration	(34)

\* significantly different from pre-exercise, P<0.05; Post=immediately post-exercise; NC=no change; GSH=reduced glutathione; GSSG=oxidised glutathione; GSH-Px=erythrocyte glutathione peroxidase; SOD= erythrocyte superoxide dismutase.

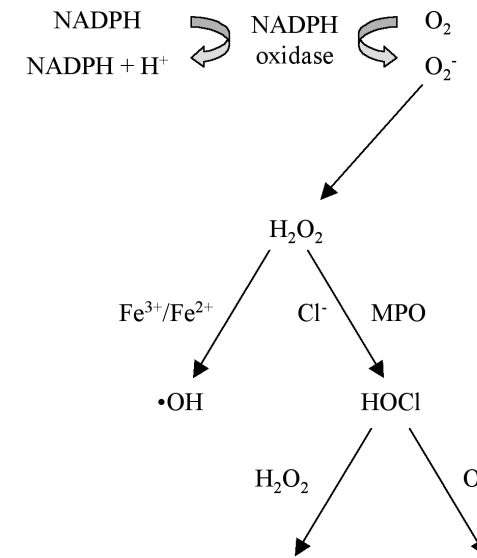
### Effects of exercise on DNA damage

The studies summarised previously in this review have investigated the possible link between neutrophil activation and oxidative stress after exercise. Other studies have examined the possible link between oxidative stress, DNA integrity and markers of DNA degradation after exercise. There are some reports of DNA strand breaks (i.e. damage) after strenuous prolonged exercise such as a half-marathon race (25) and a triathlon race (12). Tsai *et al.* also observed evidence of leukocyte DNA damage after a marathon, and this correlated with elevated plasma lipid peroxide concentrations, suggesting a link between oxidative stress and DNA damage after exercise (46).

In contrast, others have assessed DNA damage by measuring leukocyte or urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) or 7,9-dihydro-8-oxoguanine (8-oxoG). One group found reduced levels of 8-OHdG in the nucleus of lymphocytes after exercise, and proposed that this response was indicative of enhanced DNA repair mechanisms after exercise. Others have reported disparate findings. Lymphocyte 8-OHdG did not change after a single bout of downhill running (36), or across 8 days of exercise training (30). Urinary 8-OHdG has been reported to increase after an initial bout of exercise and is then maintained across subsequent days of exercise, suggesting an adaptive response to exercise (1, 30). Conversely, there was no change in urinary 8-OHdG and 8-oxoG after and ultraendurance triathlon (24) and no alteration after a bout of downhill running (36). The reasons behind this disparity have not been adequately resolved.

Therefore, because of variation in the methods used to detect DNA damage, it remains unclear whether exercise-induced oxidative stress results in DNA

damage. In one of the studies above, the authors proposed that alterations in leukocyte DNA after exercise are not the result of oxidative stress, and such alterations are unlikely to cause damage to chromosomes (12). Further studies are required to assess the effects of oxidative stress during exercise on cellular function and integrity.



### Effects of exercise on neutrophil haem-oxygenase (HO-1) protein

Neutrophils show adaptive responses to regular exercise with respect to the expression of inducible haem-oxygenase (HO-1) protein. Neutrophil HO-1 expression appears to be greatest after prolonged aerobic exercise,

Figure 1. The production of ROS by neutrophils. Modified with permission from S.F. van Eeden *et al.* J Immunol Methods: 23-43, 1999.

whereas brief maximal aerobic exercise and eccentric exercise have no effect (9). A comparison of trained versus untrained individuals revealed that neutrophil HO-1 expression at rest is higher in untrained individuals (27). These data are supported by the finding that the capacity of neutrophils to produce ROS is down-regulated by daily repetition of exercise (42, 45), suggesting the existence of endogenous adaptive mechanisms. Niess *et al.* originally proposed that cytokine-mediated production of ROS could stimulate HO-1 expression in neutrophils (27). However, a subsequent study by the same group indicated that supplementation with 500 I.U. vitamin E per day for 8 days before exhaustive treadmill exercise did not influence increases in neutrophil HO-1 expression (28). The results of this latter study suggest that ROS may not be a stimulus for increased expression of HO-1 in neutrophils after exercise. Therefore, future studies could investigate the stimuli contributing to enhanced neutrophil expression of HO-1 following intense aerobic exercise.

### Summary

In summary, data from existing findings in this area suggest that post-exercise increases in neutrophil ROS production *in vitro* may challenge endogenous antioxidant defence mechanisms. Current evidence appears to indicate that ROS produced by activated neutrophils are unlikely to overwhelm endogenous antioxidants and contribute to significant levels of oxidative stress, at least in the circulation. Endogenous antioxidants may have less effect on neutrophil activation within muscle tissue. The interaction between antioxidants and neutrophil-derived ROS may be quite specific, and limited to certain biochemical pathways. The antioxidant *N*-acetylcysteine appears to be effective at attenuating neutrophil ROS production *in vitro* after maximal exercise. Vitamin C has less impact during prolonged submaximal exercise, whereas it may promote oxidative stress following eccentric exercise. It remains to be determined if increased production of ROS during exercise has implications for normal neutrophil function and maintenance of health in athletes. There are several possible avenues for future research in this area. Firstly, future studies could examine whether *N*-acetylcysteine and zinc supplementation affects neutrophil ROS production *in vitro* and HO-1 expression after submaximal exercise, and whether vitamin C is more effective in response to maximal exercise. Secondly, future studies may consider setting up an *in vitro* experiment to determine differences in plasma antioxidants and lipid markers of oxidative stress before and after exposure to activated neutrophils. Thirdly, because endogenous antioxidants act synergistically *in vivo*, future studies could examine the relative contribution of individual antioxidants, and also whether antioxidant supplements work more effectively on their own, or in combination. Lastly, from a practical standpoint, it may be important to establish prescription guidelines for the types of exercise that might promote excessive neutrophil activation and oxidative stress.

### References

1. Almar M, Villa JG, Cuevas MJ, Rodriguez-Marroyo JA, Avila C and Gonzalez-Gallego J. Urinary levels of 8-hydroxydeoxyguanosine as a marker of oxidative damage in road cycling. *Free Radic Res* 36: 247-253, 2002.

2. Anderson R and Lukey P. A biological role for ascorbate in the selective neutralisation of extracellular phagocyte-derived oxidants. *Annals of the New York Academy of Sciences* 498: 229-247, 1987.
3. Anderson R, Smit M, Joone G and van Staden A. Vitamin C and cellular immune functions. *Ann NY Acad Sci* 587: 33-48, 1989.
4. Aoi W, Naito Y, Takanami Y, Kawai Y, Sakuma K, Ichikawa H, Yoshida N and Yoshikawa T. Oxidative stress and delayed-onset muscle damage after exercise. *Free Radic Biol Med* 37: 480-487, 2004.
5. Cannon J, Orencole S, Fielding R, Meydani M, Meydani S, Fiatarone M, Blumberg J and Evans W. Acute phase response in exercise: interaction of age and vitamin E on neutrophils and muscle enzyme release. *Am J Physiol* 259: R1214-R1219, 1990.
6. Childs A, Jacobs C, Kaminski T, Halliwell B and Leeuwenburgh C. Supplementation with vitamin C and N-acetyl-cysteine increases oxidative stress in humans after an acute muscle injury induced by eccentric exercise. *Free Radic Biol Med* 31: 745-753, 2001.
7. Droge W. Free radicals in the physiological control of cell function. *Physiol Rev* 82: 47-95, 2002.
8. Dwenger A, Funck M, Lueken B, Schweitzer G and Lehmann U. Effect of ascorbic acid on neutrophil functions and hypoxanthine/xanthine oxidase-generated, oxygen-derived radicals. *Eur J Clin Chem Clin Biochem* 30: 187-191, 1992.
9. Fehrenbach E, Niess AM, Passet F, Sorichter S, Schwirtz A, Berg A, Dickhuth HH and Northoff H. Influence of different types of exercise on the expression of haem oxygenase-1 in leukocytes. *J Sports Sci* 21: 383-389, 2003.
10. Goetzl EJ. Vitamin E modulates the lipoygenation of arachidonic acid in leukocytes. *Nature* 288: 183-185, 1980.
11. Halliwell B. Free radicals, antioxidants and human disease: curiosity, cause or consequence? *Lancet* 344: 721-724, 1994.
12. Hartmann A, Pfueller S, Dennog C, Germadnik D, Pilger A and Speit G. Exercise-induced DNA effects in human leukocytes are not accompanied by increased formation of 8-hydroxy-2'-deoxyguanosine or induction of micronuclei. *Free Radic Biol Med* 24: 245-251, 1998.
13. Hasegawa H, Suzuki K, Suzuki K, Nakaji S and Sugawara K. Effects of zinc on the reactive oxygen species generating capacity of human neutrophils and on the serum opsonic activity *in vitro*. *Luminescence* 15: 321-327, 2000.
14. Hessel E, Haberland A, Muller M, Lerche D and Schimke I. Oxygen radical generation of neutrophils: a reason for oxidative stress during marathon running? *Clin Chim Acta* 298: 145-156, 2000.
15. Huupponen M, Makinen L, Hyvonen P, Sen C, Rankinen T, Vaisanen S and Rauramaa R. The effect of N-acetylcysteine on exercise-induced priming of human neutrophils. *Int J Sport Med* 16: 399-403, 1995.
16. Jacob R. Vitamin C. In: *Modern Nutrition in Health and Disease*, edited by Shils M, Olson J and Shike M. Hagerstown, MD: Williams & Wilkins, 1994, p. 432-448.
17. Konig D, Wagner K-H, Elmadfa I and Berg A. Exercise and oxidative stress: significance of antioxidants with reference to inflammatory, muscular, and systemic stress. *Exerc Immunol Rev* 7: 108-133, 2001.
18. Krause R, Patruta S, Daxbock F, Fladerer P, Biegelmayer C and Wenisch C. Effect of vitamin C on neutrophil function after high-intensity exercise. *Eur J Clin Invest* 31: 258-263, 2001.

19. Macha M, Schlafer M and Kluger M. Human neutrophil hydrogen peroxide generation following physical exercise. *J Sport Med Phys Fitness* 30: 412-419, 1990.
20. Mastaloudis A, Morrow J, Hopkins D, Devaraj S and Traber M. Antioxidant supplementation prevents exercise-induced lipid peroxidation, but not inflammation, in ultramarathon runners. *Free Radic Biol Med* 15: 1329-1341, 2004.
21. Miyazaki H, Oh-ishi S, Ookawara T, Kizaki T, Toshinai K, Ha S, Haga S, Ji L and Ohno H. Strenuous endurance training in humans reduces oxidative stress following exhausting exercise. *Eur J Appl Physiol* 84: 1-6, 2001.
22. Nielsen HB, Kharazmi A, Bolbjerg ML, Poulsen HE, Pedersen BK and Secher NH. N-acetylcysteine attenuates oxidative burst by neutrophils in response to ergometer rowing with no effect on pulmonary gas exchange. *Int J Sports Med* 22: 256-260, 2001.
23. Nieman D, Henson D, Butterworth D, Warren B, Davis J, Fagoaga O and Nehlsen-Cannarella S. Vitamin C supplementation does not alter the immune response to 2.5 hours of running. *Int J Sport Nutr* 7: 173-184, 1997.
24. Nieman DC, Henson DA, McAnulty SR, McAnulty L, JD M, Ahmed A and Heward C. Vitamin E and immunity after the Kona triathlon world championship. *Med Sci Sports Exerc* 36: 1328-1335, 2004.
25. Niess A, Baumann M, Roecker K, Horstmann T, Mayer F and Dickhuth H. Effects of intensive endurance exercise on DNA damage in leucocytes. *J Sports Med Phys Fitness* 38: 111-115, 1998.
26. Niess AM, Dickhuth HH, Northoff H and Fehrenbach E. Free radicals and oxidative stress in exercise-immunological aspects. *Exerc Immunol Rev* 5: 22-56, 1999.
27. Niess AM, Passek F, Lorenz I, Schneider EM, Dickhuth HH, Northoff H and Fehrenbach E. Expression of the antioxidant stress protein heme oxygenase-1 (HO-1) in human leukocytes. *Free Radic Biol Med* 26: 184-192, 1999.
28. Niess AM, Sommer M, Schneider M, Angres C, Tschositsch K, Golly IC, Battenfeld N, Northoff H, Biesalski HK, Dickhuth HH and Fehrenbach E. Physical exercise-induced expression of inducible nitric oxide synthase and heme oxygenase-1 in human leukocytes: effects of alpha-tocopherol supplementation. *Antioxid Redox Signal* 2: 113-126, 2000.
29. Ohman L, Dahlgren C, Follin P, Lew D and Stendahl O. N-acetylcysteine enhances receptor-mediated phagocytosis by human neutrophils. *Agents Actions* 36: 271-277, 1992.
30. Okamura K, Doi T, Hamada K, Sakurai M, Yoshioka Y, Mitsuzono R, Migita T, Sumida S and Sugawa-Katayama Y. Effect of repeated exercise on urinary 8-hydroxy-deoxyguanosine excretion in humans. *Free Radic Res* 26: 507-514, 1997.
31. Peake JM. Exercise-induced alterations in neutrophil degranulation and respiratory burst activity: possible mechanisms of action. *Exerc Immunol Rev* 8: 49-100, 2002.
32. Peake JM, Gerrard D and JFT Griffin. Plasma zinc and immune markers in runners in response to a moderate increase in training volume. *Int J Sports Med* 24: 212-216, 2003.
33. Pizza FX, Cavender D, Stockard A, Baylies H and Beighle A. Anti-inflammatory doses of ibuprofen: effect on neutrophils and exercise-induced muscle injury. *Int J Sports Med* 20: 98-102, 1999.
34. Quindry J, Stone W, King J and Broeder C. The effects of acute exercise on neutrophils and plasma oxidative stress. *Med Sci Sports Exerc* 35: 1139-1145, 2003.
35. Robson P, Bouic P and Myburgh K. Antioxidant supplementation enhances neutrophil oxidative burst in trained runners following prolonged exercise. *Int J Sp Nutr Ex Metab* 13: 369-381, 2003.
36. Satchek JM, Milbury PE, Cannon JG, Roubenoff R and Blumberg JB. Effect of vitamin E and eccentric exercise on selected biomarkers of oxidative stress in young and elderly men. *Free Radic Biol Med* 34: 1575-1588, 2003.
37. Sen C, T R, S V and Raurama R. Oxidative stress following human exercise: effect of N-acetylcysteine supplementation. *J Appl Physiol* 76: 2570-2577, 1994.
38. Singh A, Failla M and Deuster P. Exercise-induced changes in immune function: effects of zinc supplementation. *J Appl Physiol* 76: 2298-2303, 1994.
39. Smith J, Telford R, Mason I and Weidemann M. Exercise, training and neutrophil microbicidal activity. *Int J Sport Med* 11: 179-187, 1990.
40. Stagnaro R, Pierrri I, Piovano P, Baracco F, De Palma M and Indiveri F. Thiol containing antioxidant drugs and the human immune system. *Bull Eur Physiopathol Respir* 23: 303-307, 1987.
41. Steensberg A, JD M, Toft AD, Bruunsgaard H and Pedersen BK. Prolonged exercise, lymphocyte apoptosis and F2-isoprostanes. *Eur J Appl Physiol* 87: 38-42, 2002.
42. Suzuki K, Naganuma S, Totsuka M, Suzuki KJ, Mochizuki M, Shiraisi M, Nakaji S and Sugawara K. Effects of exhaustive endurance exercise and its one-week daily repetition on neutrophil count and functional status in untrained men. *Int J Sports Med* 17: 205-212, 1996.
43. Suzuki K, Nakaji S, Yamada M, Liu Q, Kurakake S, Okamura N, Kumae T, Umeda T and Sugawara K. Impact of a competitive marathon race on systemic cytokine and neutrophil responses. *Med Sci Sports Exerc* 35: 348-355, 2003.
44. Suzuki K, Sato H, Kikuchi T, Abe T, Nakaji S, Sugawara K, Totsuka M, Sato K and Yamaya K. Capacity of circulating neutrophils to produce reactive oxygen species after exhaustive exercise. *J Appl Physiol* 81: 1213-1222, 1996.
45. Suzuki K, Totsuka M, Nakaji S, Yamada M, Kudoh S, Liu Q, Sugawara K, Yamaya K and Sato K. Endurance exercise causes interaction among stress hormones, cytokines, neutrophil dynamics, and muscle damage. *J Appl Physiol* 86: 1360-1367, 1999.
46. Tsai K, Hsu TG, Hsu KM, Cheng H, Liu TY, Hsu CF and Kong CW. Oxidative DNA damage in human peripheral leukocytes induced by massive aerobic exercise. *Free Radic Biol Med* 31: 1465-1472, 2001.
47. Voisin C, Aerts C and Wallaert B. Prevention of in vitro oxidant-mediated alveolar macrophage injury by cellular glutathione and precursors. *Bull Eur Physiopathol Respir* 23: 309-313, 1987.
48. Weiss S. Tissue destruction by neutrophils. *New Eng J Med* 320: 365-379, 1989.