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Ultra-Endurance Exercise and Oxidative Damage

Implications for Cardiovascular Health

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Abstract

At least 30 minutes of moderate-intensity physical activity accumulated on most, preferably all days is considered the minimum level necessary to reduce the risk of developing cardiovascular disease. Despite an unclear explanation, some epidemiological data paradoxically suggest that a very high volume of exercise is associated with a decrease in cardiovascular health. Although ultra-endurance exercise training has been shown to increase antioxidant defences (and therefore confer a protective effect against oxidative stress), an increase in oxidative stress may contribute to the development of atherosclerosis via oxidative modification of low-density lipoprotein (LDL). Research has also shown that ultra-endurance exercise is associated with acute cardiac dysfunction and injury, and these may also be related to an increase in free radical production. Longitudinal studies are needed to assess whether antioxidant defences are adequate to prevent LDL oxidation that may occur as a result of increased free radical production during very high volumes of exercise. In addition, this work will assist in understanding the accrued effect of repeated ultra-endurance exercise-induced myocardial damage.

A central cause of cardiovascular disease (CVD) is atherosclerosis, where the diameter of blood vessels is reduced by deposits of lipids and other substances.[1,2] Whilst significant progress has been made in recent years to improve cardiovascular health, CVD still represents the greatest health and financial burden for developed countries. Health professionals have long recognised that inactivity is a major modifiable risk factor associated with the progression of atherosclerosis and, therefore, CVD. Physical activity guidelines generally recommend, based on substantial evidence,[3,4] that individuals should accumulate at least 30 minutes of moderate-intensity physical activity each day to improve and maintain their health.[5] Indeed, research has shown an increased life expectancy in world-class non-ultra-endurance athletes.[6] Paradoxically, however, epidemiological data suggest that a very high volume of exercise is associated with an increase in the risk of developing CVD.[7,8] At present, epidemiological data do not reveal a clear link between long-term, large-volume training and an enhanced susceptibility to CVD. In the context of these data, the aim of this article is to outline the mechanisms for exercise-induced oxidative stress, evaluate the relationship between oxidative stress, exercise and cardiovascular health and to consider whether elevated oxidative stress due to the high volume of exercise may explain these findings.

1. Reactive Oxygen Species (ROS) and Oxidative Damage

Reactive oxygen species (ROS) is the term given to free radicals and non-radical compounds derived from oxygen that have the ability to modify lipids, proteins, carbohydrates and nucleic acids resulting in what is collectively called oxidative stress or oxidative damage to these compounds.[9] Free radicals are highly reactive molecules or ions that contain at least one unpaired electron in their outer orbital or valence shell.[10] Examples of free radicals include the superoxide anion, the hydroxyl radical and the hypochlorous radical. Hydrogen peroxide (H_2O_2) is an example of a ROS that is not a free radical.

2. Mechanism of Exercise-Induced ROS Production and Oxidative Damage

Exercised-induced ROS production is believed to be due to electron 'leakage' at the mitochondria and the increased activity of metabolic processes and immune responses that result in increased ROS. The magnitude of the increased ROS during exercise has been reported to be between 2–10%.[11]

Most types of exercises are accompanied by an increase in oxygen uptake and an increased flow of electrons through the electron transport chain. Oxidative phosphorylation occurs at the inner membrane of the mitochondria where single electrons are passed to the hydrogen-oxygen intermediates. This process results in small amounts of O_2 being incompletely reduced, which leads to an increased production of superoxide radicals (O_2^-). These radicals are further reduced to H_2O_2, hydroxy radicals ($OH^{\bullet-}$) and ultimately to water.[12]

ROS can also result from the activity of xanthine oxidase, an enzyme that is present in most human tissues (including skeletal muscle). Xanthine oxidase functions as a catalyst in a series of reactions converting hypoxanthine to uric acid.[13] Under normal conditions, 80–90% of xanthine oxidase exists as dehydrogenase,[14,15] which uses nicotinamine adenine dinucleotide (NAD) as an electron acceptor.[13] However, during exercise, it is converted to its oxidase form[16] and in this form it uses molecular O_2 instead of NAD as an electron acceptor. Molecular O_2 is therefore reduced and the superoxide radical is formed.[17] The stimulus for this to occur during exercise seems to be proteolysis caused by the disturbances in intracellular calcium homeostasis, heat stress and oxidation of the thiol groups.[18]

Several cells in the immune system are also capable of forming significant quantities of ROS. Neutrophils, monocytes and macrophages are all activated in response to exercise[19,20] and these cells form ROS to eliminate infectious agents and initiate the inflammatory response to exercise-induced injury in muscle.[21,22] The activity of ROS producing immune cells, such as neutrophils, is dependent upon a number of hormones and cytokines,[23] which act in

response to the intensity and duration of an exercise bout.[24,25] Once signals have been received from various chemoattractants, neutrophils move from the blood to local inflammatory sites.[26] Having arrived at the site of injury and infection, chemoattractants are released by the neutrophils in order to recruit additional neutrophils and a variety of other mononuclear cells to the local area. However, a problem with this process is that neutrophils have a poor capacity to distinguish between foreign and host antigens, and depend on elements of the immune system to identify the appropriate antigen.[27] Indeed, if target selection processes are not stringently controlled, neutrophils may release their toxic agents on normal host tissues resulting in inflammation and oxidative stress.[26]

In the normal process of phagocytosis or engulfment of the bacteria or tissue fragments, activation of the respiratory burst and degranulation of cytoplasmic granules occur as coordinated events that contribute to the cytotoxic capacity of the neutrophil.[26] The respiratory burst involves a sudden stimulus-induced increase in non-mitochondrial oxidative metabolism, which results in the production of the superoxide anion and associated ROS.[28]

Although iron is not a source of free radicals, it does, however, play a role in ROS production. Free iron enables the superoxide radical to be converted to the extremely reactive hydroxyl radical.[12] Under normal circumstances, iron remains bound to iron transporters and iron-dependent proteins; however, under conditions related to exercise, iron can be mobilised to assist in this reaction. It has been proposed that the mobilisation of iron from ferritin can occur with the assistance of xanthine oxidase, through the actions of superoxide anions.[29,30] Given that the production of xanthine oxidase is reported to increase during periods of metabolic stress[16] and certainly in response to eccentric exercise,[31] it is possible that this may increase the mobilisation of iron. In addition, the high destruction rates of red blood cells observed in high impact endurance sports may increase levels of free iron[12] and contribute to the conversion of superoxide radicals to the more reactive hydroxyl radical via the iron-catalysed Haber-Weiss reaction.[32,33]

3. Minimising Oxidative Stress: the Antioxidant Defence System

An antioxidant is any substance that reduces the extent of oxidative damage by creating a less active radical or by reducing the damaging free radical chain reaction.[34] The antioxidant defence system includes both endogenous and exogenous (dietary) antioxidants. Endogenous antioxidants include the enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and glutathione (GSSH). The processes by which these antioxidants can reduce the damage inflicted by ROS vary greatly. SOD catalyses the reaction of the superoxide radical to H_2O_2,[35] while CAT removes the H_2O_2 by converting it to H_2O and O_2. GPX catalyses the reduction of H_2O_2 and fatty acid peroxides to H_2O and oxidised glutathione, using a reduced form of glutathione as the electron donor.[36] Heat shock proteins (HSP) also provide a protective effect by their ability to maintain the viability, function and proliferative capacity of immunocompetent cells during and after exercise. In particular, the HSP haeme oxygenase-1, assists in cell protection from oxidative stress by decreasing the intracellular pool of free iron, which subsequently decreases the generation of ROS via the Fenton reaction.[37,38]

Dietary antioxidants include, but are not limited to, α-tocopherol (vitamin E), ascorbic acid (vitamin C), coenzyme Q_{10} and β-carotene (pro-vitamin A) and each exerts its own protective effect. The lipid-soluble antioxidant vitamin E is believed to be the most important and effective nutritional antioxidant in the important lipid phases of the cell, as it contributes to membrane stability and fluidity by preventing lipid peroxidation.[13] Research has shown that enrichment of endothelial cells and smooth muscle cells with vitamin E, or β-carotene inhibits the ability of the cells to modify low-density lipoprotein (LDL)[39] and therefore reducing the potential development of atherosclerosis. Vitamin C is the most abundant exogenous antioxidant found in plasma and interstitial fluids[40] and it protects against lipid

peroxidation in plasma.[40] Vitamin C also interacts with the vitamin E radical to recycle it back to vitamin E. Coenzyme Q_{10} is a lipid-soluble antioxidant that acts as an electron carrier in the mitochondria.[41] It is at least as effective, if not more effective, as vitamin E in the prevention of peroxidative damage as it scavenges peroxyl radicals generated within the liposomal membranes.[41,42] Finally, the major carotenoid precursor to vitamin A, β-carotene, is the most efficient quencher of singlet oxygen.

4. Direct Evidence of Exercise-Induced Oxidative Damage

Davies et al.[43] investigated whether exhaustive exercise in rats resulted in an increase in free radical concentration in the liver and muscle and caused subsequent oxidative damage. A 2- to 3-fold increase in muscle and liver free radical production was reported by the authors, who used signals from electron paramagnetic resonance (EPR) spectroscopy to quantify free radical production in whole tissue and tissue homogenates. Recently in humans, incremental knee-extensor exercise was performed at increasing work rates, while free radical production across the active muscle bed was determined by measuring α-phenyl-tert-butylnitrone adducts using EPR.[44] Results showed that exercise increased the net outflow of adducts as well as lipid hydroperoxides (indirect marker of oxidative stress) in a progressive manner consistent with the increase in work rate. This provides direct evidence that there is an intensity-dependent increase in free radical production from exercising muscle.

5. Limitations of the Indirect Measurement of Oxidative Damage

A plethora of studies have investigated the effects of exercise on markers of oxidative damage. Before reviewing some of these, consideration of the limitations of indirect measures of oxidative damage is warranted. The quantification of oxidative damage is usually carried out using two approaches: (i) the assessment of the concentration of oxidative by-products in body fluids or expired gas; or (ii) the evaluation of the oxidative potential (e.g. susceptibility of plasma/lipids to oxidise) *ex vivo*. Oxidative by-products of lipid oxidation include exhaled alkane, pentane and ethane gases and circulating, urinary or tissue concentrations of compounds such as conjugated dienes, aldehydes, lipid hydroperoxides or isoprostanes.[45] Many of these measures have been criticised for their questionable accuracy and validity.[46-48]

One of the most common measures of lipid oxidation is the thiobarbituric acid reactive substances (TBARS) technique. The method involves reacting biological samples with thibarbituric acid (TBA) and measuring the production of the formed complex. Malondialdehyde (MDA) is a stable by-product of lipid peroxidation produced from the oxidation of polyunsaturated fatty acids[49] and is known to bind to TBA. A measure of this complex is regarded as an indirect measure of MDA. A number of researchers claim that the TBARS method works well when it is applied to defined membrane systems such as microsomes and liposomes, but when applied to tissue extracts and body fluids, a number of problems can potentially emerge[45] and has therefore been criticised for its lack of specificity, sensitivity and reproducibility.[50] Currently, techniques such as the direct detection of MDA using high-performance liquid chromatography (HPLC) or the measurement of isoprostanes (breakdown products of arachidonic acid metabolism) are recognised as more valid measures of lipid peroxidation in human biological samples such as blood and urine.

6. Indirect Evidence of Exercise-Induced Oxidative Damage

The relationship between exercise and oxidative stress has been examined in many studies.[51-54] One of the first investigations was published in the late 1970s; it was reported that in untrained humans, cycling at 75% of maximal oxygen uptake ($\dot{V}O_{2max}$), expired pentane concentration was almost double the resting value.[55] Consistent with this finding was the work by Pincemail et al.[56] who found an increase in expired pentane concentration four times the resting value in five healthy male

subjects cycling at 45%, 60% and 75% of $\dot{V}O_{2max}$. Lovlin et al.[57] investigated the effects of varying intensities on MDA production using TBARS in a group of six untrained males.[57] They reported that intermittent bouts of exercise at 40% and 70% of $\dot{V}O_{2max}$ caused a significant decrease in plasma MDA concentration. However, as exercise intensity increased, MDA levels started to rise until, following the final exhaustive exercise bout, MDA showed a significant increase. The authors concluded that free radical production was generated by exhaustive maximal exercise whilst short periods of submaximal exercise seemed to inhibit lipid peroxidation.[57] An alternative explanation is that antioxidant defences are sufficient to meet an increase in ROS production during low-intensity exercise but that as exercise intensity increases, these defences are surpassed.

Maughan et al.[58] examined the effect of a 5-minute treadmill run (horizontal) at 75% of age-predicted heart rate maximum followed by a 45-minute downhill run at that same speed on the serum TBARS concentration. No change was reported to have occurred immediately post-exercise; however, 6 hours later, a significant increase in serum TBARS concentration was reported. Although difficult to interpret, the authors suggest that this was due, at least in part, to the loss of membrane integrity in association with free radical reactions.

In summary, research has shown that irrespective of the type of indirect measures of oxidative stress that, in general, acute bouts of endurance exercise at a moderate to high intensity increases oxidative stress.

7. Low-Density Lipoprotein Oxidation and Exercise

Oxidative transformation of trapped LDL is believed to occur in two stages. The first begins prior to the recruitment of monocytes to the site of injury and ends with the oxidation of the lipid component of the LDL. The second stage begins with the recruitment of monocytes to the lesion where they are converted into macrophages, which results in further modification of LDL.[59,60] The mechanism for LDL oxidation is believed to be the same at rest as it is in exercise. Importantly, however, the rate increases with exercise.[61] Oxidative stress and the subsequent oxidation of LDL is considered a major contributor to the impairment of endothelial function[62] and the development of atherosclerotic lesions.[63,64] Research has shown that acute bouts of exercise can increase markers of LDL oxidation.[65-68] Therefore, it is plausible that high volumes of energy expenditure from ultra-endurance exercise may advance the progression in atherogenesis as a result of the increase in free radical production, oxidative stress and LDL oxidation.

8. Exercise/Physical Activity, Energy Expenditure and Morbidity

Numerous prospective epidemiological studies have investigated the effects of exercise or estimates of physical activity (derived from energy expenditure data) on mortality.[69,70] The consistent conclusion from these investigations is that low levels of exercise/physical activity are strongly associated with increased mortality independent of other factors.[69-71] However, a closer look at data presented from some of these studies reveals a number of interesting features. In one of the first studies to describe the relationship between energy expenditure and mortality, Paffenbarger et al.[3] divided 16 936 individuals involved in the Harvard Alumni Health Study into eight groups based on activity-derived energy expenditure. They reported a decline in the relative risk of death with increasing physical activity up to the group that had an energy expenditure >14 700 kJ/week (figure 1). In this group, the relative risk of death increased to 0.62 which was the same as group 5 (8400–10 499 kJ/week) and only 0.01 better than group 4 (6300–8399 kJ/week). There was no mention as to whether the increased risk of death in the highest energy expenditure group was statistically greater than the preceding groups. The same cohort of subjects had physical activity energy expenditure and mortality data analysed 10 years later with similar findings; a nonsignificant increase in the age-standardised mortality rate in subjects reporting energy expenditure >14 700 kJ/

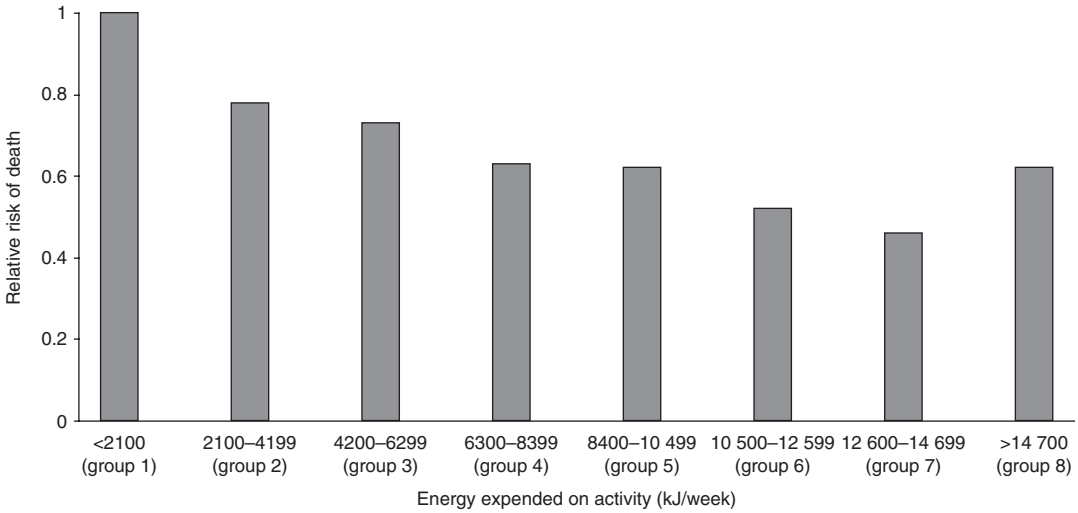

Fig. 1. Relative risk of death corresponding to energy expenditure.

week (figure 2).[7] In addition, if the activity was vigorous, the nonsignificant increase in mortality occurred when energy expenditure was >12 600 kJ/week. The authors note that this finding is similar to that reported in the British Regional Heart Study conducted on 7735 men aged 40–59 years where vigorously active men had higher rates of heart attacks than men performing moderate or moderately vigorous activity.[72] One explanation for this outcome may be an elevation in oxidative stress stemming from increased physical activity.[61]

Quinn et al.[8] looked at relationships between caloric expenditure and mortality in 348 subjects from the Michigan State University Longevity Study. Six caloric groups were established based on the amount of energy expended per week on aerobic activities such as bicycling, rowing, jogging and swimming. As expected, death rates were highest in the two lowest caloric expenditure groups (36.5% for group 1 = 0 kJ/week and 41.7% for group 2 = 1–1679 kJ/week). However, death rates were higher in group 6 than groups 3, 4 or 5 (24.6% for group 6 = >10 500 kJ/week; 13.8% for group 5 = 6300–10 499; 21.4% for group 4 = 3780–6299 kJ/week and 24.3% for group 3 = 1680–3779 kJ/week). The investigators also analysed data from subjects who reported CVD, and CVD was the highest in groups 1 and 6 (figure 3).

9. Ultra-Endurance Athletes

Ultra-endurance athletes are a unique group to investigate a potential relationship between long-term, high-energy expenditure and cardiovascular health. Coyle[73] defined endurance exercise as typically being maintained for durations of between 30 minutes and 4 hours. However, many ultra-endurance athletes train for, and compete in, events such as Ironman triathlon, cycling races or ultra-marathon running that can last up to 30 hours. It should also be noted that the number of individuals training for, and competing in, these types of events is continually increasing as evidenced by the number of new races of these types being established each year. Athletes in these events usually train between 20–40 hours/week and expend over 70 000 kJ/week during training.[74]

10. Ultra-Endurance Exercise and Cardiovascular Health

Several studies have provided evidence of at least cardiac dysfunction following running races lasting between 19 and 30 hours[75] and as a result of Ironman triathlons.[76-78] Douglas et al.[79] showed that ultra-endurance training produces moderate left ventricular hypertrophy and the same authors

showed a significantly increased prevalence of mitral and tricuspid regurgitation.[80]

Laslett et al.[75] investigated the effect of ultra-endurance running up to a distance of 161km (100 miles) on the production of cardiac troponin T (cTnT), a marker of myocardial damage. With a small sample of five athletes, a marked elevation in serum levels of cTnT levels was reported in response to the ultra-endurance running race. The levels of cTnT ranged from 0.29 to 8.55 ng/mL, which is considerably higher than the level associated with myocardial injury (0.2 ng/mL). Consistent with this finding, Rifai et al.[77] also found a significant increase ($p < 0.002$) in cTnT in 23 athletes as a result of an Ironman triathlon. In addition, the authors reported that the cardiac ejection fraction significantly decreased ($p < 0.002$) in response to the race. Neumayr et al.[81] analysed serum levels of cardiac troponin I (cTnI), recognised to be a more sensitive and specific marker of cardiomyocyte necrosis than cTnT, and found elevated levels in 13 of 38 participants in an ultra-endurance cycling race. Unfortunately, the investigation did not include an untrained control group to allow for a meaningful comparison of differences between the two groups in a rested condition. Finally, in a recent investigation by La Gerche et al.,[78] using only elite ultra-endurance triathletes to increase the sensitivity of the study, minimal evidence of cardiac dysfunction post-triathlon resulted. No change was detected in specific post-race markers (cTnI) related to cardiac damage. Moreover, only one subject showed a change in their post-race ECG, which returned to normal within 25 days. This study shows that any damage that may occur as a result of acute ultra-endurance activity may be transient; however, it is unclear what the accumulative effect of many acute ultra-endurance exercise bouts will have on the cardiovascular system.

Although the cause of cardiac damage resulting from very high volume exercise is unknown, it has been speculated that oxidative stress may be involved.[82] Indeed, Corretti et al.[83] showed that glycolytic inhibition occurred simultaneously with an increase in Ca^{2+} concentration in response to free radical generation (hydroxyl) in rabbit hearts. Subsequently, it was hypothesised that free radicals inhibited glycolysis, causing a perturbation in Ca^{2+} homeostasis, leading to excessive Ca^{2+} and dysfunction.[83]

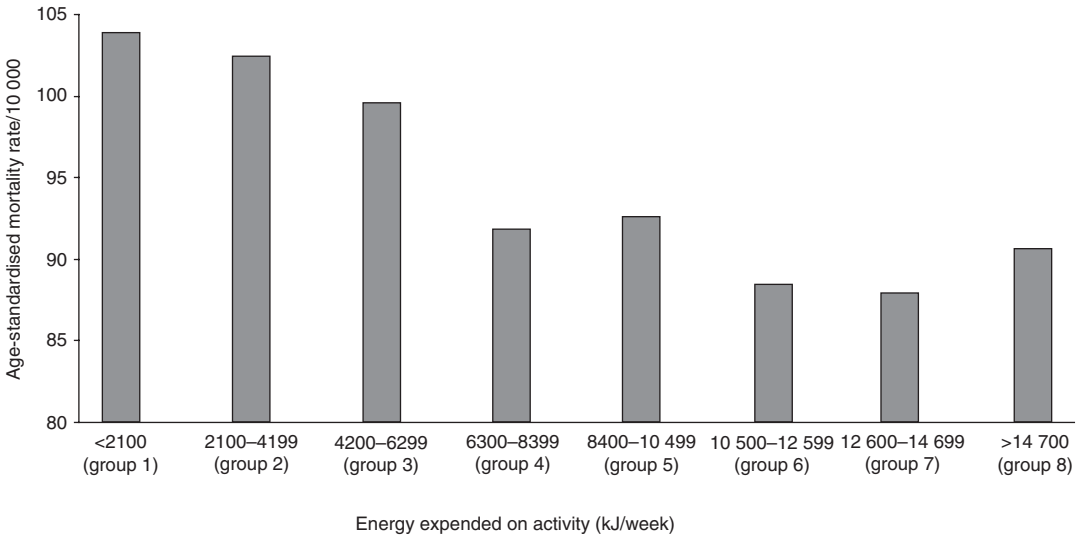

Fig. 2. Age-standardised mortality rate/10 000 corresponding to energy expenditure.

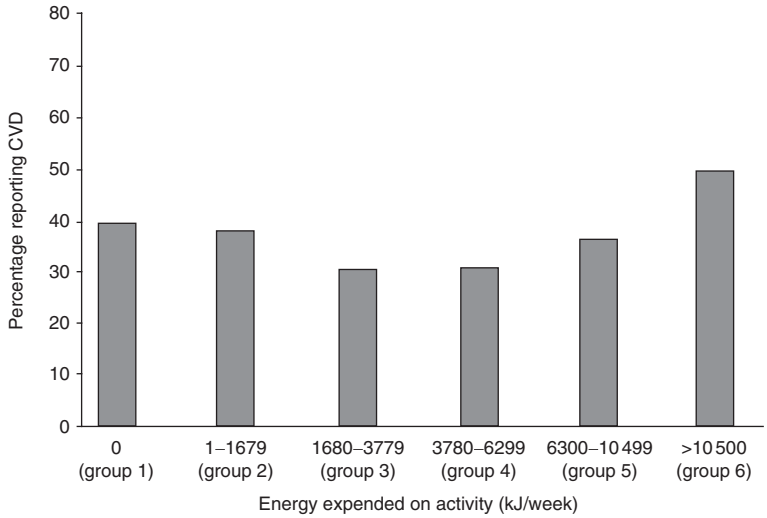

Fig. 3. Percentage of participants reporting cardiovascular disease (CVD) corresponding to energy expenditure.

11. Ultra-Endurance Exercise and Oxidative Damage

There are a number of excellent papers reviewing exercise and oxidative stress;[61,84-86] however, this article focuses on ultra-endurance exercise, which we have defined as continuous exercise maintained for durations >4 hours. From the literature we have identified a number of studies that have investigated the topic of ultra-endurance exercise and oxidative damage.

Kanter et al.[87] examined the influence of an 80km ultra-marathon on changes in various physiological markers in nine males who ran an average of 121 km/week. Blood was sampled before and after the race and results showed that the exercise produced a significant increase in MDA (using TBARS) and creatine kinase leading the authors to suggest that muscle damage may be related to exercise-induced lipid peroxidation.

Mastaloudis et al.[68] sought to determine the effect of a 50km ultra-marathon race on biomarkers of oxidative stress in a group of athletes who trained an average running distance of 49.9km. Although the training volume completed by athletes in this study was considerably less than previous research,[87] the results were consistent with a significant increase in F_2-isoprostances reported post-race compared with pre-race blood samples.

Sanchez-Quesada et al.[65] also investigated the effect of long duration aerobic exercise on the susceptibility of LDL to oxidation. Six well trained male marathon and ultra-marathon runners with an average weekly training distance of between 75 and 135 km/week participated in the study. The subjects ran continuously for 4 hours at an average speed of 11.95 km/hour; mean energy expenditure during exercise was estimated to be 3400 kcal. Blood was sampled and various markers were compared pre- and post-exercise. Prior to exercise, subjects fasted for at least 10 hours; however, during the run, they were allowed to consume food and beverages *ad libitum*. Analysis of the blood revealed an increase in the susceptibility of LDL to oxidation, as measured by conjugated dienes.

Not all studies have found high volumes of exercise to result in oxidative stress. For example, Margaritis et al.[88] reported no evidence of oxidative stress following an ultra-endurance triathlon; concentrations of disulfide glutathione and TBARS did not significantly change as a result of exercise. This finding was consistent with data from an earlier ultra-endurance study conducted by Ginsburg et al.,[89] who reported a decrease in the susceptibility

of lipids to peroxidise in response to an Ironman triathlon, as measured by conjugated dienes.

Inconsistencies between the studies reviewed may be explained by differences in the exercise demands (e.g. intensity, type, duration, training protocols and dietary status) and the training status of the participants. Indeed, it has been suggested that in response to a single bout of exercise, there is an intensity below which oxidative stress does not occur.[61] Furthermore, there is evidence to suggest that excessive production of free radicals occurs only when the exercise is exhaustive.[90] However, exercise duration is not a variable that resolves the inconsistencies. This is highlighted by research that has found a significant increase,[87] a decrease[89] and no change[88] in the production of oxidative stress in response to ultra-endurance exercise. Also, as discussed previously, differences in findings between studies may also be related to the methods used by researchers to detect oxidative stress.

Collectively considered, however, the limited research has generally shown that high volume exercise increases ROS production and various markers of oxidative stress. Given that the oxidative modification hypothesis predicts that oxidation of LDL is an early event in atherosclerosis that contributes to atherogenesis,[91] it certainly seems plausible that participating in long-term ultra-endurance exercise that significantly increases markers of LDL oxidation, negative health implications may result unless exercise-induced pro-oxidant activity is neutralised or balanced by adaptations in ultra-endurance athletes.

12. Exercise Training, Adaptations and Oxidative Stress

It is well known that regular exercise produces various adaptations that reduce oxidative stress.[84,92] A potential explanation of the disparity in results reported in the literature on ultra-endurance exercise and oxidative stress may be due to inter-individual differences in the antioxidant capacity or the responses to oxidative stress. These differential responses may be due to differences in the physiological adaptations to exercise training.

Exercise training is accompanied by an increase in the activities of key antioxidant enzymes.[93,94] In addition, an increase in mitochondrial volume in response to endurance training results in a relatively lower oxidative load, which may attenuate the generation of ROS.[95] Exercise may also assist in a combative role against ROS damage that can occur during exhaustive exercise by decreasing the loosely bound iron in muscles.[96]

Immunological changes are also likely as a result of exercise training; trained individuals are less likely to experience localised inflammation in exercised muscles. Training also strengthens muscle fibres and protects against muscle damage.[97] Furthermore, the neutrophils of trained individuals have a reduced capacity to produce microbiocidal ROS.[98] Therefore, in a single bout of exercise, the magnitude of oxidative stress may be strongly influenced by an individual's training history.

A small number of studies have examined adaptations of the antioxidant system to ultra-endurance exercise. Recently, we examined changes in various antioxidants in athletes training for half- and full-distance Ironman triathlons. In half-distance Ironman athletes we found that 13 athletes exercising for 14.5 hours/week had significantly higher GPX activity compared with age-, sex- and weight-matched controls (unpublished data). In addition, and with a different cohort of 26 athletes who were training 17 (±3.4) hours a week for a full Ironman triathlon, we found significantly higher activities of GPX and CAT compared with age-, sex- and mass-matched controls (unpublished data). Our data collectively confirm that high volumes of exercise are also associated with elevated antioxidant defences against oxidative damage and that training status may influence the magnitude of adaptation of these defences. However, it is unclear whether this result translates into an improved cardiovascular health status.

13. Exogenous Antioxidants, Ultra-Endurance Exercise and Oxidative Stress

Oxidative stress that results from exercise can potentially be minimised by dietary antioxidants

such as vitamin C, vitamin E and β-carotene. The two most investigated nutritional antioxidants in the area of exercise-induced oxidative stress have been vitamin C and vitamin E. Most of the studies investigating the effect of supplementation on the production of oxidative stress in endurance exercise have shown that either there is no change in oxidative stress (with supplementation) or that there is an attenuation in its production from pre- to post-measurement.[99,100]

Unfortunately, few studies have examined the relationship between vitamin supplementation and oxidative stress with ultra-endurance exercise. One study found that an increase in the susceptibility of lipids to peroxidise following an Ironman triathlon was unrelated to antioxidant use or levels of vitamins A, C or E.[89] In contrast, in an investigation by Mastaloudis et al.,[101] 22 ultra-endurance runners were supplemented with 1000mg of vitamin C and 300mg of α-tocopherol or a placebo for 3 weeks prior to a 50km ultra-endurance marathon. Data showed a significant increase in the concentration of F_2-isoprostanes in pre-race compared with post-race blood samples in the placebo group ($p < 0.001$). In addition, the concentration of F_2-isoprostanes was significantly higher in the placebo group compared with those supplementing.

Inconsistent with this, is the study by Nieman et al.[54] who investigated 38 ultra-endurance athletes who received 800 IU/day of vitamin E or placebo capsules for 2 months prior to competing in an Ironman triathlon. The data showed a significant interaction ($p < 0.05$) as plasma F_2-isoprostanes increased nearly 2-fold in the vitamin E supplementation group compared with the placebo group. Nieman et al.[102] also investigated the effect of vitamin C supplementation on oxidative stress and immune changes in 15 ultra-endurance runners compared with 13 runners who received placebo supplementation. During the 7 days prior to the race and on race day, runners received either 1500 mg/day of vitamin C or a placebo. Blood was sampled 1 hour prior to race start, at the 32km mark and at the completion of the ultra-endurance marathon race (mean of 69km). The data showed that despite the increased concentration of ascorbic acid in the supplementation group, there were no significant group or interaction effects in lipid hydroperoxides, F_2-isoprostane or in a number of immune markers.

Although the limited research that has examined the influence of antioxidant supplementation on oxidative stress resulting from endurance exercise has generally produced similar findings, data related to ultra-endurance exercise, antioxidant supplementation and oxidative stress are less consistent. Indeed, previously discussed problems in relation to methodology may in part explain differences in the results. In addition, the amount of vitamins consumed by athletes is not always reported by authors. Certainly, what is clear is that ultra-endurance exercise and its relationship to antioxidant supplementation requires further investigation.

14. Conclusion

Ultra-endurance exercise has been shown to elevate oxidative stress. Furthermore, ultra-endurance exercise is associated with acute cardiac dysfunction as evidenced by ECG abnormalities and cTnI. Increased oxidative damage has been implicated as the cause of these effects. Oxidative stress is also associated with the development of atherosclerosis and the impairment of endothelial function. Some epidemiological evidence suggests that individuals who expend large amounts of energy through exercise may be at increased risk of CVD and mortality, which may be associated with an increase in oxidative damage stemming from prolonged aerobic exercise. However, this response may be mitigated in ultra-endurance athletes as a result of exercise-induced adaptations (increased antioxidant defence, less ROS production). Therefore, despite the high-volume energy expenditure, this population of athletes may not be at a substantially greater risk of developing CVD. Further investigation is recommended to clarify the relationship between the accumulative effect of ultra-endurance exercise on oxidative stress, CVD and cardiovascular health.

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References

1. Ross R. Atherosclerosis: an inflammatory disease. N Engl J Med 1999; 340 (2): 115-26
2. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 1993; 362 (6423): 801-9
3. Paffenbarger RJ, Hyde RT, Wing AL, et al. Physical activity, all-cause mortality, and longevity of college alumni. N Engl J Med 1986; 314 (10): 605-13
4. Pate RR, Pratt M, Blair SN, et al. Physical activity and public health: a recommendation from the Centers for Disease Control and Prevention and the American College of Sports Medicine. JAMA 1995; 273 (5): 402-7
5. Australian Government, Department of Health and Ageing. An active way to better health: national physical activity guidelines for adults [online]. Available from URL: http://www.health.gov.au/internet/wcms/Publishing.nsf/Content/phd-physical-activity-adults-pdf-cnt.htm/$File/adults_phys.pdf [Accessed 2006 Apr 4]
6. Sarna S, Sahi T, Koskenvuo M, et al. Increased life expectancy of world class male athletes. Med Sci Sports Exerc 1993; 25 (2): 237-44
7. Lee IM, Hsieh CC, Paffenbarger RJ. Exercise intensity and longevity in men. The Harvard Alumni Health Study. JAMA 1995; 273 (15): 1179-84
8. Quinn TJ, Sprague HA, Van Huss WD, et al. Caloric expenditure, life status, and disease in former male athletes and nonathletes. Med Sci Sports Exerc 1990; 22 (6): 742-50
9. Dhalla NS, Temsah RM, Netticadan T. Role of oxidative stress in cardiovascular diseases. J Hypertens 2000; 18 (6): 655-73
10. Sen CK. Oxidants and antioxidants in exercise. J Appl Physiol 1995; 79 (3): 675-86
11. Boveris A, Chance B. The mitochondrial generation of hydrogen peroxide: general properties and effect of hyperbaric oxygen. Biochem J 1973; 134 (3): 707-16
12. Sjodin B, Hellsten Westing Y, Apple FS. Biochemical mechanisms for oxygen free radical formation during exercise. Sports Med 1990; 10 (4): 236-54
13. Niess AM, Dickhuth HH, Northoff H, et al. Free radicals and oxidative stress in exercise: immunological aspects. Exerc Immunol Rev 1999; 5: 22-56
14. Bindoli A, Cavallini L, Rigobello MP, et al. Modification of the xanthine-converting enzyme of perfused rat heart during ischemia and oxidative stress. Free Radic Biol Med 1988; 4 (3): 163-7
15. Parks DA, Williams TK, Beckman JS. Conversion of xanthine dehydrogenase to oxidase in ischemic rat intestine: a reevaluation. Am J Physiol 1988; 254 (5 Pt 1): G768-74
16. Corte ED, Stirpe F. The regulation of rat liver xanthine oxidase: involvement of thiol groups in the conversion of the enzyme activity from dehydrogenase (type D) into oxidase (type O) and purification of the enzyme. Biochem J 1972; 126 (3): 739-45
17. Kuppusamy P, Zweier JL. Characterization of free radical generation by xanthine oxidase: evidence for hydroxyl radical generation. J Biol Chem 1989; 264 (17): 9880-4
18. Parks DA, Granger DN. Xanthine oxidase: biochemistry, distribution and physiology. Acta Physiol Scand Suppl 1986; 548: 87-99
19. Bury TB, Pirnay F. Effect of prolonged exercise on neutrophil myeloperoxidase secretion. Int J Sports Med 1995; 16 (6): 410-2
20. Bousquet J, Chanez P, Mercier J, et al. Monocytes, exercise, and the inflammatory response. Exerc Immunol Rev 1996; 2: 35-44
21. Evans WJ, Cannon JG. The metabolic effects of exercise-induced muscle damage. Exerc Sport Sci Rev 1991; 19: 99-125
22. Woods JA, Davis JM. Exercise, monocyte/macrophage function, and cancer. Med Sci Sports Exerc 1994; 26 (2): 147-56
23. Smith JA. Neutrophils, host defense, and inflammation: a double-edged sword. J Leukoc Biol 1994; 56 (6): 672-86
24. McCarthy DA, Dale MM. The leucocytosis of exercise: a review and model. Sports Med 1988; 6 (6): 333-63
25. Gabriel HH, Heine G, Kroger K, et al. Exercise and atherogenesis: where is the missing link? Exerc Immunol Rev 1999; 5: 96-102
26. Pyne DB. Regulation of neutrophil function during exercise. Sports Med 1994; 17 (4): 245-58
27. Weiss SJ. Tissue destruction by neutrophils. N Engl J Med 1989; 320 (6): 365-76
28. Halliwell B, Gutteridge JM. Oxygen free radicals and iron in relation to biology and medicine: some problems and concepts. Arch Biochem Biophys 1986; 246 (2): 501-14
29. Thomas CE, Morehouse LA, Aust SD. Ferritin and superoxide-dependent lipid peroxidation. J Biol Chem 1985; 260 (6): 3275-80
30. Biemond P, Swaak AJ, Beindorff CM, et al. Superoxide-dependent and -independent mechanisms of iron mobilization from ferritin by xanthine oxidase: implications for oxygen-free-radical-induced tissue destruction during ischaemia and inflammation. Biochem J 1986; 239 (1): 169-73
31. Hellsten Y, Frandsen U, Orthenblad N, et al. Xanthine oxidase in human skeletal muscle following eccentric exercise: a role in inflammation. J Physiol 1997; 498 (Pt 1): 239-48
32. Haber F, Weiss J. The catalytic decomposition of hydrogen peroxide by iron salts. Proc R Soc Lond 1934; 147: 332-51
33. McCord JM, Day ED. Superoxide-dependent production of hydroxyl radical catalyzed by iron-EDTA complex. FEBS Lett 1978; 86 (1): 139-42
34. Goldfarb AH. Nutritional antioxidants as therapeutic and preventive modalities in exercise-induced muscle damage. Can J Appl Physiol 1999; 24 (3): 249-66
35. Camhi SL, Lee P, Choi AM. The oxidative stress response. New Horiz 1995; 3 (2): 170-82
36. Flohe L. Role of GSH peroxidase in lipid peroxide metabolism. In: Yagi K, editor. Lipid peroxidation in biology and medicine. New York: Academic, 1982: 6377-81
37. Stocker R. Induction of haem oxygenase as a defence against oxidative stress. Free Radic Res Commun 1990; 9 (2): 101-12
38. Marini M, Frabetti F, Musiani D, et al. Oxygen radicals induce stress proteins and tolerance to oxidative stress in human lymphocytes. Int J Radiat Biol 1996; 70 (3): 337-50
39. Navab M, Imes SS, Hama SY, et al. Monocyte transmigration induced by modification of low density lipoprotein in cocultures of human aortic wall cells is due to induction of monocyte chemotactic protein 1 synthesis and is abolished by high density lipoprotein. J Clin Invest 1991; 88 (6): 2039-46

40. Frei B, England L, Ames BN. Ascorbate is an outstanding antioxidant in human blood plasma. Proc Natl Acad Sci U S A 1989; 86 (16): 6377-81
41. Frei B, Kim MC, Ames BN. Ubiquinol-10 is an effective lipid-soluble antioxidant at physiological concentrations. Proc Natl Acad Sci U S A 1990; 87 (12): 4879-83
42. Stocker R, Bowry VW, Frei B. Ubiquinol-10 protects human low density lipoprotein more efficiently against lipid peroxidation than does alpha-tocopherol. Proc Natl Acad Sci U S A 1991; 88 (5): 1646-50
43. Davies KJ, Quintanilha AT, Brooks GA, et al. Free radicals and tissue damage produced by exercise. Biochem Biophys Res Commun 1982; 107 (4): 1198-205
44. Bailey DM, Young IS, McEneny J, et al. Regulation of free radical outflow from an isolated muscle bed in exercising humans. Am J Physiol Heart Circ Physiol 2004; 287 (4): H1689-99
45. Halliwell B, Chirico S. Lipid peroxidation: its mechanism, measurement, and significance. Am J Clin Nutr 1993; 57 (5 Suppl.): 715S-24S
46. Springfield JR, Levitt MD. Pitfalls in the use of breath pentane measurements to assess lipid peroxidation. J Lipid Res 1994; 35 (8): 1497-504
47. Jenkins RR. Exercise and oxidative stress methodology: a critique. Am J Clin Nutr 2000; 72 (2 Suppl.): 670S-4S
48. Janero DR. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radic Biol Med 1990; 9 (6): 515-40
49. Wade CR, van Rij AM. Plasma malondialdehyde, lipid peroxides, and the thiobarbituric acid reaction. Clin Chem 1989; 35 (2): 336
50. Meagher EA, FitzGerald GA. Indices of lipid peroxidation in vivo: strengths and limitations. Free Radic Biol Med 2000; 28 (12): 1745-50
51. Ayres S, Baer J, Subbiah MT. Exercised-induced increase in lipid peroxidation parameters in amenorrheic female athletes. Fertil Steril 1998; 69 (1): 73-7
52. Almar M, Villa JG, Cuevas MJ, et al. Urinary levels of 8-hydroxydeoxyguanosine as a marker of oxidative damage in road cycling. Free Radic Res 2002; 36 (3): 247-53
53. Orhan H, van Holland B, Krab B, et al. Evaluation of a multiparameter biomarker set for oxidative damage in man: increased urinary excretion of lipid, protein and DNA oxidation products after one hour of exercise. Free Radic Res 2004; 38 (12): 1269-79
54. Nieman DC, Henson DA, McAnulty SR, et al. Vitamin E and immunity after the Kona Triathlon World Championship. Med Sci Sports Exerc 2004; 36 (8): 1328-35
55. Dillard CJ, Litov RE, Savin WM, et al. Effects of exercise, vitamin E, and ozone on pulmonary function and lipid peroxidation. J Appl Physiol 1978; 45 (6): 927-32
56. Pincemail J, Camus G, Roesgen A, et al. Exercise induces pentane production and neutrophil activation in humans. Effect of propranolol. Eur J Appl Physiol Occup Physiol 1990; 61 (3-4): 319-22
57. Lovlin R, Cottle W, Pyke I, et al. Are indices of free radical damage related to exercise intensity. Eur J Appl Physiol Occup Physiol 1987; 56 (3): 313-6
58. Maughan RJ, Donnelly AE, Gleeson M, et al. Delayed-onset muscle damage and lipid peroxidation in man after a downhill run. Muscle Nerve 1989; 12 (4): 332-6
59. Sparrow CP, Parthasarathy S, Steinberg D. A macrophage receptor that recognizes oxidized low density lipoprotein but not acetylated low density lipoprotein. J Biol Chem 1989; 264 (5): 2599-604
60. Brown MS, Goldstein JL. Atherosclerosis: scavenging for receptors. Nature 1990; 343 (6258): 508-9
61. Alessio HM. Exercise-induced oxidative stress. Med Sci Sports Exerc 1993; 25 (2): 218-24
62. Ogita H, Liao J. Endothelial function and oxidative stress. Endothelium 2004; 11 (2): 123-32
63. Steinberg D, Parthasarathy S, Carew TE, et al. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. N Engl J Med 1989; 320 (14): 915-24
64. Witztum JL. The oxidation hypothesis of atherosclerosis. Lancet 1994; 344 (8925): 793-5
65. Sanchez-Quesada JL, Homs-Serradesanferm R, Serrat-Serrat J, et al. Increase of LDL susceptibility to oxidation occurring after intense, long duration aerobic exercise. Atherosclerosis 1995; 118 (2): 297-305
66. Wetzstein CJ, Shern-Brewer RA, Santanam N, et al. Does acute exercise affect the susceptibility of low density lipoprotein to oxidation? Free Radic Biol Med 1998; 24 (4): 679-82
67. Liu ML, Bergholm R, Makimattila S, et al. A marathon run increases the susceptibility of LDL to oxidation in vitro and modifies plasma antioxidants. Am J Physiol 1999; 276 (6 Pt 1): E1083-91
68. Mastaloudis A, Leonard SW, Traber MG. Oxidative stress in athletes during extreme endurance exercise. Free Radic Biol Med 2001; 31 (7): 911-22
69. Paffenbarger RJ, Brand RJ, Sholtz RI, et al. Energy expenditure, cigarette smoking, and blood pressure level as related to death from specific diseases. Am J Epidemiol 1978; 108 (1): 12-8
70. Villeneuve PJ, Morrison HI, Craig CL, et al. Physical activity, physical fitness, and risk of dying. Epidemiology 1998; 9 (6): 626-31
71. Lee IM, Paffenbarger RJ. Physical activity and stroke incidence: the Harvard Alumni Health Study. Stroke 1998; 29 (10): 2049-54
72. Shaper AG, Wannamethee G, Weatherall R. Physical activity and ischaemic heart disease in middle-aged British men. Br Heart J 1991; 66 (5): 384-94
73. Coyle EF. Physiological determinants of endurance exercise performance. J Sci Med Sport 1999; 2 (3): 181-9
74. O'Toole ML, Douglas PS, Hiller WD. Lactate, oxygen uptake, and cycling performance in triathletes. Int J Sports Med 1989; 10 (6): 413-8
75. Laslett L, Eisenbud E, Lind R. Evidence of myocardial injury during prolonged strenuous exercise. Am J Cardiol 1996; 78 (4): 488-90
76. Douglas PS, O'Toole ML, Hiller WD, et al. Cardiac fatigue after prolonged exercise. Circulation 1987; 76 (6): 1206-13
77. Rifai N, Douglas PS, O'Toole M, et al. Cardiac troponin T and I, echocardiographic [correction of electrocardiographic] wall motion analyses, and ejection fractions in athletes participating in the Hawaii Ironman Triathlon. Am J Cardiol 1999; 83 (7): 1085-9
78. La Gerche A, Boyle A, Wilson AM, et al. No evidence of sustained myocardial injury following an Ironman distance triathlon. Int J Sports Med 2004; 25 (1): 45-9
79. Douglas PS, O'Toole ML, Hiller WD, et al. Electrocardiographic diagnosis of exercise-induced left ventricular hypertrophy. Am Heart J 1988; 116 (3): 784-90
80. Douglas PS. Cardiac considerations in the triathlete. Med Sci Sports Exerc 1989; 21 (5 Suppl.): S214-8

81. Neumayr G, Gaenzer H, Pfister R, et al. Plasma levels of cardiac troponin I after prolonged strenuous endurance exercise. Am J Cardiol 2001; 87 (3): 369-71, A10

82. Starnes JW, Bowles DK. Role of exercise in the cause and prevention of cardiac dysfunction. Exerc Sport Sci Rev 1995; 23: 349-73

83. Corretti MC, Koretsune Y, Kusuoka H, et al. Glycolytic inhibition and calcium overload as consequences of exogenously generated free radicals in rabbit hearts. J Clin Invest 1991; 88 (3): 1014-25

84. Radak Z, Taylor AW, Ohno H, et al. Adaptation to exercise-induced oxidative stress: from muscle to brain. Exerc Immunol Rev 2001; 7: 90-107

85. Banerjee AK, Mandal A, Chanda D, et al. Oxidant, antioxidant and physical exercise. Mol Cell Biochem 2003; 253 (1-2): 307-12

86. Urso ML, Clarkson PM. Oxidative stress, exercise, and antioxidant supplementation. Toxicology 2003; 189 (1-2): 41-54

87. Kanter MM, Lesmes GR, Kaminsky LA, et al. Serum creatine kinase and lactate dehydrogenase changes following an eighty kilometer race. Relationship to lipid peroxidation. Eur J Appl Physiol Occup Physiol 1988; 57 (1): 60-3

88. Margaritis I, Tessier F, Richard MJ, et al. No evidence of oxidative stress after a triathlon race in highly trained competitors. Int J Sports Med 1997; 18 (3): 186-90

89. Ginsburg GS, Agil A, O'Toole M, et al. Effects of a single bout of ultraendurance exercise on lipid levels and susceptibility of lipids to peroxidation in triathletes. JAMA 1996; 276 (3): 221-5

90. Sastre J, Asensi M, Gasco E, et al. Exhaustive physical exercise causes oxidation of glutathione status in blood: prevention by antioxidant administration. Am J Physiol 1992; 263 (5 Pt 2): R992-5

91. Stocker R, Keaney Jr JF. Role of oxidative modifications in atherosclerosis. Physiol Rev 2004; 84 (4): 1381-478

92. Alessio HM, Goldfarb AH. Lipid peroxidation and scavenger enzymes during exercise: adaptive response to training. J Appl Physiol 1988; 64 (4): 1333-6

93. Holloszy JO, Booth FW. Biochemical adaptations to endurance exercise in muscle. Annu Rev Physiol 1976; 38: 273-91

94. Tiidus PM, Houston ME. Antioxidant and oxidative enzyme adaptations to vitamin E deprivation and training. Med Sci Sports Exerc 1994; 26 (3): 354-9

95. Moller P, Wallin H, Knudsen LE. Oxidative stress associated with exercise, psychological stress and life-style factors. Chem Biol Interact 1996; 102 (1): 17-36

96. Jenkins RR, Krause K, Schofield LS. Influence of exercise on clearance of oxidant stress products and loosely bound iron. Med Sci Sports Exerc 1993; 25 (2): 213-7

97. Ebbeling CB, Clarkson PM. Exercise-induced muscle damage and adaptation. Sports Med 1989; 7 (4): 207-34

98. Smith JA, Telford RD, Mason IB, et al. Exercise, training and neutrophil microbicidal activity. Int J Sports Med 1990; 11 (3): 179-87

99. Kanter MM, Nolte LA, Holloszy JO. Effects of an antioxidant vitamin mixture on lipid peroxidation at rest and postexercise. J Appl Physiol 1993; 74 (2): 965-9

100. Goldfarb AH, Sen CK. Antioxidant supplementation and the control of oxygen toxicity during exercise. In: Sen CK, Packer L, Hanninen O, editors. Exercise and oxygen toxicity. Amsterdam: Elsevier Science, 1994: 163-89

101. Mastaloudis A, Morrow JD, Hopkins DW, et al. Antioxidant supplementation prevents exercise-induced lipid peroxidation, but not inflammation, in ultramarathon runners. Free Radic Biol Med 2004; 36 (10): 1329-41

102. Nieman DC, Henson DA, McAnulty SR, et al. Influence of vitamin C supplementation on oxidative and immune changes after an ultramarathon. J Appl Physiol 2002; 92 (5): 1970-7

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