Blood cells as functional markers of antioxidant vitamin status

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Antioxidants have shown beneficial effects in several biological systems, in which they were able to prevent oxidative stress-associated damage. Vitamins C and E are key antioxidants in humans. Dietary intake cannot accurately reflect plasma vitamin levels. However, the plasma levels of antioxidant vitamins could also reflect the acute assimilation of these vitamins. It has been pointed out that antioxidant vitamin blood contents reach a saturation level by intake of dietary supplements. Antioxidant vitamin plasma levels are the parameter most used to determine antioxidant status. However, the vitamin plasma levels may not reflect the nutritional status of vitamins. It has been pointed out that the vitamin E in adipose tissue can be used as a measure of vitamin E status. To determinate antioxidant vitamin contents in lymphocytes and neutrophils after exercise is a useful tool to assess the functional status of antioxidant vitamins.

Antioxidants: Vitamin E: Vitamin C: Oxidative stress: Exhaustive exercise

Oxidative stress is believed to be an important causative factor in human ageing and in the development of chronic disease, operating through the formation and effect of oxidatively damaged macromolecules or their degradation products. Several pathophysiological mechanisms are known to cause an overproduction of reactive oxygen species (ROS), including exposure to transition metal ions and activation of polymorphonuclear neutrophils (PMN) and macrophages leading to generation of superoxide anion and hypochlorite (Li, 1999).

Many antioxidants have shown beneficial effects in different biological systems, in which they were able to prevent oxidative stress-associated damage. Epidemiological evidence indicates that high plasma concentrations of exogenous antioxidants are associated with lower risk of cardiovascular disease and several types of cancer (Morrisey & Sheehy, 1999; Padayatty et al. 2003). This important function of antioxidant vitamins means that adequate indicators of the situation of antioxidant vitamins depots and availability must be found.

A varied and balanced diet should provide adequate amounts of all nutrients. Vitamins C and E are key antioxidants which in humans are exclusively obtained from the diet. Vitamin C is hydrophilic and protects water-soluble components of the body, while vitamin E is a lipid-soluble antioxidant which protects cell membranes from peroxidative damage. There is evidence that these key antioxidants may interact in vivo. Vitamin C in the aqueous phase is capable of recycling lipid-bound vitamin E. Antioxidant vitamin status depends not only on vitamin intake, but it can also be influenced by other factors such as exercise, smoking habits and levels of other nutrients in the diet which may influence the absorption and metabolism of antioxidant vitamins (Hamilton et al. 2000; Traber et al. 2001b; Aguilo et al. 2003). Therefore, dietary intake cannot accurately reflect plasma vitamins levels. However, the plasma levels of antioxidant vitamins could also reflect the acute assimilation of these vitamins.

Functional foods to increase the antioxidant vitamins bioavailability

In the scientific arena, there is some controversy about how much antioxidant vitamins are required to avoid suboptimal supply and deficiency (Food and Nutrition Board, 2000; Horwitt, 2001; Traber, 2001a), or to avoid oxidative damage in people engaged in physical activity (Packer & Obermüller-Jevic, 2002). Conflicting results from vitamin E intervention studies suggest supplemental vitamin E malabsorption, but also that bioavailability of vitamin E increases with rich-fat meal (Leonard et al. 2004), and absorption of vitamin E depends on an individual’s ability to absorb fat, and to obtain maximal absorption, vitamin E must be given at meals (Iuliano et al. 2001). To overcome these problems, new functional foods have been designed to enhance the antioxidant vitamins bioavailability (Pons et al. 2002).

It is well known that oranges contain a high concentration of vitamin C (50 mg vitamin C/100 g) and that almonds contain a high concentration of vitamin E (20 mg vitamin E/100 g) (Feinberg et al. 1995; Mataix et al. 2003; Moreiras et al. 2003). To assess the contribution of an isotonic orange and almond-based beverage (Pons et al. 2002) as a vehicle to increase the bioavailability of vitamin E, female voluntary subjects received a 250 mg a-tocopherol acetate capsule together with 500 ml isotonic almond beverage (n 10) or 500 ml mineral water (n 9). There were no differences between groups in anthropometric characteristics (body mass index: 23·1 ± 1·2 kg/m²) in daily dietary intake. Blood
samples were taken in basal conditions and 4h after the supplementation to determine vitamin E, glucose, total protein, uric acid, total and direct bilirubin and triglyceride plasma levels. Insulin serum levels were determined under basal conditions and also 1, 2, and 4h after the supplementation. The subjects who had taken the vitamin E capsule with the orange and almond-based beverage showed higher plasma levels of vitamin E (+33 %) and vitamin E/VLDL-cholesterol ratio (+64 %), 4h after the supplementation, than those that had taken water (Fig. 1); both values within the normal range. The orange and almond-based beverage was found to have no effect on blood metabolites. An increase in insulin levels was observed only 1h after the orange and almond-based beverage was taken, probably due to the energy and nutrient content of the orange and almond-based beverage. A single dose of vitamin E was enough to increase plasma levels of this vitamin. Therefore, the orange and almond-based beverage used increased vitamin E bioavailability. It contained 203 kJ/100 ml, 19% lipid, 68% total sugar, 10% protein, 1.8 mg/100 ml calcium, 4.2 mg/100 ml magnesium, 12.8 mg/100 ml sodium, 33.7 mg/100 ml potassium, 69.7 μg/100 ml iron, and 44.5 μg/100 ml zinc, and only traces of vitamins C and E. The orange and almond-based beverage can be then enriched with additional vitamins C and E to attain good antioxidant vitamin blood levels (Cases et al. 2005).

**Blood cells as biomarkers of vitamins C and E**

Vitamin C concentration in plasma is tightly controlled by mediated tissue transport, absorption and excretion. Plasma vitamin C concentration about 60–70 μmol/l has been used as indicative of good dietary intake. Immune blood cells, such as lymphocytes and neutrophils, contain 1–4 nmol concentration of vitamin C and saturate at vitamin C doses between 100 and 200 mg daily. Lymphocytes are reported to be saturated at plasma concentrations of >50 μmol/l. Thus, cells are saturated before plasma. When plasma vitamin C contents approach maximal concentration, additional vitamin C is lost in urine. Vitamin C doses higher than 200 mg daily would not be necessary to avoid oxidative damage in non-risk groups in the population (Padayatty et al. 2003). Thus, the lymphocyte or neutrophil vitamin C concentration is a good marker of the vitamin C status.

Plasma levels of vitamin E is the most used biomarker to assess vitamin E status and is the one for which most data are available. A daily dietary intake of about 15–30 mg α-tocopherol is required to maintain plasma optimal levels. This amount of vitamin E could be obtained from dietary sources if a concerted effort was made to eat foods high in vitamin E. In contrast, the amounts of supplemental vitamin E suggested as protective from epidemiological studies are many times higher than those that could be obtained from the diet. The correlation between α-tocopherol and blood lipids, especially cholesterol, is very strong. Consequently, it is recommended that plasma α-tocopherol concentrations be lipid corrected. Plasma vitamin E is quickly redistributed between tissues, mainly in the adipose tissue, and it has been indicated that no reflect the vitamin E status. For this reason, it has been pointed out that the adipose tissue vitamin E could be used as an useful measure of vitamin E status (Kayden et al. 1983).

During exercise, there is increased mitochondrial respiration, allowing for greater ROS production through the incomplete reduction of oxygen to water. In response to exercise-induced muscle damage, neutrophils and macrophages migrate to the site, infiltrate the muscle tissue, activate cytokines, and produce additional ROS. Excess generation of ROS may overwhelm natural cellular antioxidant defences leading to lipid peroxidation and further contributing to muscle damage. Thus, there is an apparent paradox between the benefits of moderate and the damaging strenuous exercise. Sportsmen who practise exhaustive exercise present increased oxidative stress risk, and increased demand for antioxidant vitamins. The higher ROS production induced by exercise could use the vitamin E depots in tissues in order to counteract this ROS production. Then, exercise is a good model to establish the importance of plasma vitamin E to reflect the tissue status for this vitamin. In a previous work we demonstrated that the recommended daily intake of vitamin E is insufficient to avoid the oxidative stress induced by intense exercise (a mountain stage) in professional cyclists (Cases et al. 2003). The plasma levels of vitamin E falls to values below basal levels after intense exercise as a mountain cyclist stage (Aguiló et al. 2005).

The dose levels of antioxidants administered seems to be important to decrease the deleterious effects induced by exercise. Administration of 330 mg/d of vitamin E decreases oxidative stress markers after intensive aerobic training in cyclists (Rokitzki et al. 1994). Administration of lower doses of vitamin E (20 mg/d α-tocopheryl succinate) and ascorbic acid (120 mg/d) for 4 weeks to triathletes decreases muscle damage (Palazzetti et al. 2004).

In a previous study, we evaluated the effects of vitamin C diet supplementation on plasma and lymphocyte levels of this vitamin after repetitive episodes of hypoxia-reoxygenation induced by diving apnea (Sureda et al. 2004). Seven voluntary male professional apnea divers participated in this study. The sportsmen were divided randomly into two groups. One group was supplemented with vitamin C capsules (1 g/d) for 7 days, and the other group took a placebo. This study was a double-blind cross study. Ten days after the first diving apnea session wash-out period, we repeated
the procedure, but changing the diet-supplemented capsules, i.e. the first group supplemented with vitamin C, was now supplemented with placebo. The subjects practised diving in apnea for about 4 h remained intermittently more than 1 h without breathing and under hypoxic conditions. After the supplementation, placebo and supplemented group had similar plasma and lymphocyte basal values of vitamin C. Plasma vitamin C increased only in the supplemented group after diving. Also, the vitamin C concentration in lymphocytes increased after diving apnea but the increase was significant only in the supplemented group.

In other work (Cases et al. 2005), we studied the combined effects of 1 month antioxidant vitamins C and E supplementation on exercise-induced oxidative stress. Fourteen male trained amateur runners (age, 34.5 ± 3.6 years; body mass index, 23.1 ± 0.6 kg/m2) volunteered to take part in this study. They all trained 7.5 ± 1.3 h each week. The subjects took neither antioxidant dietary supplement nor any routine medication for 1 month prior to the study. Antioxidant vitamin intake in the supplemented group was 60 ± 1 mg/d for vitamin E and 277 ± 30 mg/d for vitamin C, whereas in the placebo group vitamin E intake was 14.8 ± 1.2 mg/d and vitamin C was 162 ± 29 mg/d. We used antioxidant doses that can be provided by a diversified and well-balanced diet. After 1 month, subjects participated in a half marathon race (21 km run). The athletes took a mean ± SEM of 91 ± 10 min to finish the race. Basal plasma and lymphocyte levels of vitamin C and E after supplementation were unchanged. However, plasma vitamin C concentration increased (+30 %) after exercise only in the supplemented group and returned to basal values after 3 h recovery, whereas vitamin E remained at basal values. Lymphocytes in the supplemented group had increased (+40 %) vitamin C content after the exercise and remained high after the short recovery period. Lymphocyte vitamin C contents maintain basal levels in the placebo group. After the exercise (half-marathon), vitamin E levels in lymphocytes and neutrophils of the supplemented subjects were practically twice the levels before exercise and these levels remained high after recovery. The increase was higher in the supplemented group (about 130 %) than in the placebo (about 100 %). Neutrophils vitamin E content of the placebo group were close to those in plasma. However, the contribution of neutrophils and lymphocytes to vitamin E blood contents is of low importance, because most of the vitamin E blood content came from plasma.

The higher antioxidant vitamin availability allows the lymphocytes to increase their antioxidant defences in order to avoid the ROS deleterious effects induced by intense exercise. Immune blood cells accumulate vitamin E to prevent auto-oxidative processes, and therefore maintain their functionality. These results show that antioxidant vitamins exert a protective effect on oxidative stress on human cells, but also that the intense exercise promotes mechanisms to accumulate antioxidant vitamins into cells sensitive to the effects of ROS.

Conclusions
Our findings suggest that the determination of antioxidant vitamin content in lymphocytes and neutrophils after exercise is a useful tool to assess the functional status of antioxidant vitamins in both individuals and populations, specially among sportmen.

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References
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