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Does physical exercise modify antioxidant requirements?

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Physical training is known to induce a biochemical adaptive response which might require an increase in the ingestion and/or the absorption of micronutrients. A question that is still being raised is whether acute or chronic exercise modifies antioxidant requirements. First, the present review brings to light the most crucial studies on the topic. Second, it interprets the established relationships between antioxidant micronutrient intakes and the adaptive response of antioxidant systems. Finally, it exposes the major questions connected with antioxidant micronutrient requirements for athletes. To this effect, the training-load interaction with nutrition is taken into account. As oxidative stress cannot be avoided, the imbalance between oxidants and antioxidants can be alleviated to minimise oxidative damage and outcomes. There is growing evidence that one specific antioxidant cannot by itself prevent oxidative stress-induced damage, as direct adverse effects of supplementation are attributed to undesirable synergic effects. Other effects can be supposed that limit the endogenous adaptive effect of training. High doses of antioxidant supplements can minimise the effects of radical oxygen species themselves or generate pro-oxidant effects. Effects are only exhibited when nutritional status is deficient. There are no convincing effects of supplementation in well-trained athletes. Risk/benefit analysis emerges on evidence for an unknown risk of supranutritional intakes, a supposed impairment of adaptive effects and a still unknown long-term risk. Appropriate status can be achieved by a diversified and balanced diet, adapted to specific needs, by awareness of high-density food intakes (avoiding products containing a low density of micronutrients).

Vitamins: Carotenoids: Trace elements: Oxidative stress: Supplementation: Athletes

Introduction

Antioxidant vitamins and trace element intakes are necessary to allow endogenous adaptation and to avoid excessive stress. This could induce various forms of cellular damage, the alteration of cellular functions, and, in the case of excessive stress, cellular death by apoptosis or necrosis. Nowadays, few athletes ignore that physical exercise promotes oxidative stress. Based on this, antioxidant nutrient requirements have been arousing growing interest during the past decade.

The French Food Agency has been asked to assess enriched foods for athletes. To date, the question of exercise-induced oxidative stress brings out the question of the optimal conditions for the adaptation of antioxidants. Inefficient antioxidant systems make one vulnerable to the outcome of oxidative stress. Requirements for macronutrients in adults have been widely described. However, considering micronutrients, the ones which have to be considered are those which might be ingested in an insufficient way and those which may be lost in excess during physical exercise. Another reason for considering these micronutrients is linked to the assumption that training induces a biochemical adaptive response which might require an increase in the ingestion and/or the absorption of trace elements and vitamins. Consequently, several authors have put forward the hypothesis that for some athletes, their intake of certain antioxidant micronutrients might be less than they actually need. So far, whether acute or chronic physical exercise induces a change in antioxidant and trace element requirements has not been sufficiently discussed. The question of RDA for athletes has yet to be addressed. This must be done in relation to other fields such as increased losses of micronutrients and the dietary behaviour which changes alongside the goals of the competition. The first step is to make an inventory of the questions raised and to interpret the established relationships between micronutrient intakes and the adaptive responses of the

Abbreviations: GSH-Px, glutathione peroxidase; ROS, radical oxygen species; SOD, superoxide dismutase; SR-BI, scavenger receptor class B, type I.

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antioxidant system. The second step is to expose the major questions connected with antioxidant micronutrient requirements for athletes, taking into account the training-load interaction with nutrition.

Does physical exercise modify the status of antioxidant vitamins, carotenoids and flavonoids?

Vitamin C

Due to its implication in several metabolic pathways such as carnitine, noradrenalin and collagen synthesis, as well as in antioxidant defence, it is legitimate to say that the athletes' ascorbic acid (vitamin C) requirements are greater because of a higher rate of endogenous consumption compared with individuals who are not physically active. The human diet contains both ascorbic acid and dehydroascorbic acid, the bioavailability of which is supposed to be comparable. These compounds are almost completely absorbed in the ileum by an active transport which becomes passive in the case of ingestion of a high vitamin C dose[1]. Lack of storage implies a requirement for regular dietary intake. High vitamin C intakes increase its excretion in urine, the main elimination pathway. Plasma ascorbic acid is strongly linked to vitamin C intakes – for intakes between 50 and 90 mg/d – and it is shown to be maximal for vitamin C intakes of 200 mg/d[2].

Basal concentrations of ascorbic acid in plasma in some athletes can be low and in some cases reach marginal concentrations[3–5]. These athletes are mostly those involved in team games or those undergoing unusual periods of intensive training. Due to an absence of a parallel investigation on their dietary vitamin C intakes, it becomes difficult to interpret these data. In fact, team-game athletes frequently have poor dietary food choices with vitamin C intakes <150 mg/d[6]. Moreover, excessive training is backed by the shortage in the supply of vitamin C[7]. However, with well-trained athletes, ascorbic acid status in basal conditions is normal[6]. In addition, for most cases, athletes have a higher basal ascorbic acid status than those observed in less physically active individuals[6,8,9]. Dietary intakes, without supplements, can reach 400 mg/d. The relationship between vitamin C intakes and plasma ascorbic acid concentrations is logarithmic in athletes. The optimal bioavailability of vitamin C is shown to be reached with an intake of 200 mg/d[6]. As a consequence, any supplement above this threshold of intake has no effect on vitamin C plasma concentrations[5,10,11].

Even though there is a high nutritional density in vitamin C intakes as observed amongst highly trained athletes, paradoxically these athletes are high consumers of supplements. The consumption of supplements can generate pro-oxidant effects when the intake is above 500 mg/d[12]. With well-trained athletes, supplement intakes are frequently observed and can lead to increased lipoperoxidation indices when measured at rest or shortly after exercise[13]. This pro-oxidant effect of vitamin C could be worsened by eccentric exercise due to sizeable muscle injuries induced by such[14]. This can lead to muscle damage and/or haemolysis, leading to the release of Fe, normally stored and chelated. It is conceivable that this induces radical oxygen species (ROS) generation through the Fenton reaction, the rate of which is low in normal physiological conditions. High vitamin C intake also reduces the absorption of Cu and increases Fe absorption to the detriment of intestinal absorption of Zn[15]. This accelerated rate of Fe absorption can be detrimental when Fe stores are already abundant. These Fe stores may be abundant amongst top athletes, mainly because of their current uncontrolled practice of Fe supplementation[16]. It is also questionable whether the pro-oxidant effect of high doses is due to an imbalance between ascorbic acid and dehydroascorbic acid intakes.

After intensive exercise, natural antioxidant systems, which protect neutrophils, could be depleted. To this effect, the oxidative burst will not be carried out in an effective way. Athletes, in order to avoid infections, make use of preventive and chronic high doses (>500 mg/d) of vitamin C. The supply in vitamin C may be beneficial for some subjects involved in heavy exercising while facing problems with frequent upper respiratory tract infections[17] but the supplementation in vitamin C, as of now, has generally failed to show consistent results in improving oxidative burst activity (for a review, see Moreira *et al.* [18]). Nevertheless, there is no evidence that a prolonged consistent intake of vitamin C is favourable to antioxidant system reinforcement and training adaptation. A recent study carried out by Gomez-Cabrera *et al.* [19] showed that the supply of 1 g vitamin C/d alleviates training-induced up-regulation of the mitogenic pathway and takes a toll on the improvement of the endurance capacity within a period of 8-week training done by rats which were initially sedentary. This also applies to humans. In the study by Gomez-Cabrera *et al.* [19], the expression of PPAR-γ coactivator 1, the main transcription factor of mitogenesis which has recently been shown to be a redox-sensitive process[20], was decreased with a supplementary intake in vitamin C. The study of Khassaf *et al.* [21] showed that the heat shock protein HSP70 content in a muscle has a 6-fold increase under normal resting conditions after an 8-week vitamin C intake (500 mg/d). Moreover, HSP70 muscle content is 4·5-fold higher following exercise. This is not the case under resting conditions where it has a 3·5-fold increase amongst individuals receiving a supplement of vitamin C. The results of these studies could be interpreted as a lower adaptive response to exercise due to long-term supplies. To this effect, a question is raised about the effect of exogenous antioxidant supply on the adaptive process. A recent study produced evidence on the failure of muscles to return to normal functioning after exercise following a supply of 1 g vitamin C. This takes place despite the fact that lipoperoxidation markers were concomitantly decreased[22]. Lowering oxidative stress by antioxidant supplement may hamper normal muscle recovery function which is known to depend on ROS level.

It is admitted that the needs of athletes are higher than those of less active individuals, although a well-balanced diet which covers energetic needs provides the necessary vitamin C supply (>200 mg/d). High-training-stress periods are key periods for vitamin C deficit awareness. This nutritional risk of deficit can be reduced by a regular fruit and vegetable consumption.

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Vitamin E

It is well known that muscle cell integrity requires an adequate α-tocopherol (vitamin E) status[23]. Despite the fact that in most cases the intakes of vitamin E amongst well-trained athletes are below 12 mg/d, which is the RDA for the general population, this population is not at risk of marginal status[6]. As aerobic training increases HDL concentrations, the main plasma lipoprotein carrier of α-tocopherol, α-tocopherol incorporation in tissues is favoured. Indeed, the concentration of α-tocopherol in plasma and muscles is linked to the α-tocopherol incorporation rate which depends on the quantity of specific receptors such as scavenger receptor class B, type I (SR-BI)[24,25]. These receptors are efficient vectors for α-tocopherol incorporation in muscle cells through their specific recognition of HDL[24].

One has to be cautious when interpretating plasma α-tocopherol concentrations during training periods. Indeed, the higher the intensity in training is, the higher plasma α-tocopherol concentrations are[7]. Exercise mobilises more plasma α-tocopherol and leads to an increase in circulating concentrations[26,27]. Besides, high basal values of plasma α-tocopherol can be sometimes associated with high oxidative damage indices[28]. High concentrations found in athletes can be explained by the acceleration of its turnover with exercise-induced oxidative stress[29]. Within a short recovery period, plasma α-tocopherol concentrations could remain high. Consequently, the variation in training loads must be taken into account when interpreting α-tocopherol status. With well-led aerobic training, the increase in the concentration of α-tocopherol in plasma is not so visible[26]. Women also have to be considered separately, as they have higher α-tocopherol concentrations in tissues compared with men[30,31]. They appear to be less vulnerable to an additional stress generated by low dietary vitamin E intakes[32]. Additional antioxidant protection provided by oestrogens, whose chemical properties are similar to α-tocopherol, can spare tissue α-tocopherol in situations of oxidative stress.

Even if no clinical symptoms have been shown with long-term effects of a marginal intake in vitamin E, outcomes of deficit can be mostly shown in response to an inhabitual acute or chronic physical stress such as with over-loaded training, ageing, hypoxia exposure or amenorrhoea (in women). In those situations, vitamin E supplementation can exhibit protective effects against oxidative damage of lipid and DNA[33–35]. Such protective effects of vitamin E supplementation are not shown in well-trained athletes[36]. In most cases, pharmacological doses of vitamin E supplementation have little effect on muscle concentrations since the expression of SR-BI receptors decreases in response to high muscle α-tocopherol concentrations[37].

Recent studies have shown that high-dose supplementation of α-tocopherol (536 mg/d) increases exercise-induced oxidative stress[38,39]. Indeed, the radical form of α-tocopherol can react with the peroxide form of polyunsaturated fatty acid. *In vivo*, this pro-oxidant reaction is prevented by reducing α-tocopheryl radicals with ascorbic acid (at the interface between membrane and cytosol) or with ubiquinol (in the membrane)[40] and can be achieved with a high-content antioxidant diet.

Carotenoids

Carotenoids are fat-soluble compounds contained in fruits and vegetables. Major circulating carotenoids are β-carotene, lycopene, lutein, α-carotene, β-cryptoxanthin and zeaxanthin. Mechanisms through which carotenoids neutralise free radicals have been described *in vitro*[41]. There has been evidence of the antioxidant effect of carotenoids mainly in the mechanism of the neutralisation of singlet oxygen ($^1O_2$) generated during the photo-oxidation process. Competition during carotenoid absorption and transport, as well as synergies between the two processes, are both very complex. The food matrix effect is high for these nutrients. For example, the absorption of β-carotene present in vegetables is limited by their content of lutein whose bioavailability in vegetables is 5-fold that of β-carotene[42].

During overloaded and/or inhabitual training, the concentrations of plasma β-carotene, which is transiently lowered under normal training conditions, remains low during the phase of recovery[3,26,43]. Moreover, the drop in the concentration of carotenoids with age is steeper when individuals carry out recurrent exercise[44]. This suggests that carotenoid requirements, and especially β-carotene, increase with age and physical activity. With well-trained athletes, the prevalence of marginal plasma β-carotene concentrations (2·9 % of women and 18·5 % of men) is not higher than that found in the general population[6]. However, the lower the sum total of carotenoid plasma concentration is, the higher the plasma lipoperoxidation marker[6] is.

Among the antioxidant complex supplements given to athletes highly exposed to oxidative stress – especially in extreme conditions such as cold or high altitude – those containing β-carotene or multiple carotenoids at nutritional doses fight lipoperoxidation with more efficiency in individuals with low initial antioxidant status[5,10,45]. The origin of free radical production influences the effect of ingested antioxidant vitamins and carotenoids. In LDL, α-carotene and β-carotene are the main targets of NO-induced oxidation and are, in some situations, even more oxidised than α-tocopherol[46].

There is growing evidence that one antioxidant cannot act alone to prevent oxidative damage. The pertinence of a well-balanced antioxidant diet is to date well proven. The combined action of multiple compounds derived from a diet rich in fruits and vegetables cannot be replaced by a supplementation with a single or a combination of antioxidants. Synergic effects of vitamins C and E and carotenoids[47], as well as micronutrient competitions during intestinal absorption, suggest that the best way to avoid any nutritional error is to take a close look at how food works and what it provides specifically.

Flavonoids

The major groups of flavonoids in the human diet are the flavonols, proanthocyanidins (which include catechins), isoflavonoids, flavones, and flavanones. Due to their polyphenolic nature, flavonoids (found in abundance in plants) have antioxidant effects which vary according to their nature and quantity. Although their structure is close

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to α-tocopherol, flavonoids seem to have more of a synergistic mode of action with vitamins E and C than a sparing effect in the case of deficit in vitamin E[48,49]. Flavonoids provide a protection against peroxynitrite effects by 'scavenging' its two precursors, NO and the superoxide anion[50]. However, their protective effects on the vascular system are not only related to their antioxidant capacity. Among these flavonoids, quercetin, the major representative flavonoid subclass of flavonols, catechins and epicatechins are best known for their antioxidant capacities. High amounts of quercetin are generally found in tea, apples, red wine, broccoli, citrus fruits and red fruits. Oligomeric catechins are highly concentrated in red wine, grapes, cocoa, cranberries and apples. Other polyphenols such as soya isoflavones, mostly genistein, also have *in vitro* antioxidant capacities[51]. Due to its 'oestrogen-like' effect, genistein is shown to mimic the effects of oestrogens via the activation of mitogen-activated protein kinases and NF-κB, leading to an up-regulation of the antioxidant enzyme Mn superoxide dismutase (SOD) gene expression[52].

Polyphenol pharmacokinetics are generally characterised by a plasma peak from 5–8 h after absorption, and a persistence of significant rates up to 24–36 h. With regards to quercetin, its plasma concentration peak as well as its elimination varies according to the food source[53]. In the case of repeated intakes, a plateau whose value depends on the proportion introduced is reached[53]. Determining the daily contributions remains, however, limited by the still incomplete tables of composition of food in flavonoids.

In spite of their known antioxidant effect, and unlike vitamins C and E and β-carotene research which has many clinical experiments, there has been little experimentation done with polyphenols on human subjects, including athletes. To the best of our knowledge, the study carried out by Panza *et al.* [54] stands out as the only one that has produced evidence, among the studies carried out, about the effects on indices of oxidative stress. These authors showed that a contribution in green tea (4·6 g phenolic compounds/d) increased glutathione concentrations and decreased the indices of oxidative damage on the lipids observed within 1 min and 15 min after a resistance exercise by lowering xanthine oxidase activity. Quercetin supplementation at 1 g/d in trained cyclists over a 24 d period diminished post-exercise expression of leucocyte IL-8 and IL-10 mRNA but had no effect on muscle concentrations of NF-κB[55], which ROS generated during exercise are known to serve as critical messengers to activate upstream kinases of its cascade. The antioxidant effect is suggested not to be enough to induce a decrease in NF-κB synthesis, as quercetin ingestion does not exert protection from exercise-induced oxidative stress and inflammation[56]. However, such a supplementation led to marked differences in rates of illness (5 *v.* 45 %, respectively, in the supplemented and placebo group) during the 2-week period after intensified exercise without concomitant changes in several measures of immune function[57]. Results of supplementation in clinical experiments are also inconclusive regarding isoflavone supplementation. Their effects on antioxidant systems may not be potent enough to diminish an abrupt surge of oxidative stress due to acute exercise[48]. However, it is still unknown if a chronic intake of 'oestrogen-like' isoflavones can decrease the endogenous adaptive response. Further investigation before conclusions can be reached regarding benefits in humans, and even more so in women and women athletes, is needed.

Even though the quantities generally contained in supplements do not seem to present genotoxic effects, high doses of these dietary compounds can also be toxic and mutagenic in cell-culture systems and excess consumption by mammals could cause adverse metabolic reactions[58]. Moreover, inhibitory effects of non-haeme Fe absorption have been evidenced[59]. This property is important to consider when giving dietary advice to athletes who are more susceptible to developing Fe deficiency, in particular, those involved in overloaded training and/or those having a vegetarian or vegan diet.

Whatever the case, the complementary, additive, and/or synergistic effects resulting from multiple phytochemicals found in the flavonoid-rich foods are responsible for their wide range of observed biological properties rather than these effects being due to a single constituent alone[60,61]. The studies available are not sufficient enough for the risk/benefit assessment of the supplementation of flavonoids.

Does physical activity modify the status of antioxidant trace elements?

Zinc

Zn is required for the preservation of the structural integrity of more than 2000 transcription factors and 300 enzymes[62]. Although the binding of Zn to many proteins is determinant for their function, three key signalling processes seem to be significant targets of a deficiency in Zn: the activation of NF-κB, the activation of caspase enzymes and NO signalling. Zn can stabilise membranes and is bound to protein thiol (-SH) groups protecting them from oxidation[63]. Zn is mainly contained in meat, liver, eggs, seafood and, in a lower concentration, in dairy products and cereals. Because the intestinal absorption of Zn depends on its form and on the presence of either facilitating (lactose) or inhibiting (Cu^{2+}, Fe^{2+}, phytates, Ca^{2+}) molecules, Zn bioavailability depends on the food from which it comes[64]. Plasma Zn concentrations can drop (by 50%) due to a restricted Zn absorption[65]. Excretion of Zn in faeces of the non-absorbed Zn fraction, intestinal desquamation, as well as digestive secretions, represents 10 mg/d. The main vector of Zn loss in athletes is sweat, reaching 5 mg/d when sweating is abundant. After 2 h of aerobic exercise, losses can represent respectively 9 and 8% of Zn RDA for men and women respectively[66]. Urinary loss is very low (0·3 to 0·6 mg/d). A regulation of this way of excretion does not compensate any loss by sweating[67]. Moreover, Zn loss can be greater (up to 2 mg/d) in athletes because of proteinuria which may be induced by muscle damage due to intensive physical exercise.

Plasma concentrations have to range between 12 and 18 μmol/l. There is evidence of a marginal Zn status (< 10·7 μmol/l) in 21% of athletes which could not be explained by their daily energy expenditure[68]. Semi-vegetarianism (exclusion of red meat) increases the prevalence of inadequate Zn status. This behaviour is

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frequently shown in female athletes with consequences on Zn status being reinforced due to an energy-restricted diet.

The more the subject undergoes training, the lower the plasma concentration of Zn is, and inversely, the less the subject undergoes training, the higher the plasma concentration of Zn is. This is proven in the case of interindividual approaches only[69] and not in the case of intra-individual approaches[69,70]. The concentration of erythrocyte Zn and metallothionein, a marker of the Zn reserve, is high in well-trained aerobic athletes[71]. This suggests that, in most cases, athletes are unlikely to benefit from Zn supplementation.

The increase in Zn demand due to physical activity is achieved by a well-balanced diet. If the necessity to increase Zn supplies is proven (long-lasting exercise in very hot environments could be the rare cases), it is of prime importance to pay attention to the ratio with Ca, Cu and Fe. Like other nutrients, Zn exhibits a U-shaped dose–response curve, with adverse health effects occurring when either too little or too much Zn is present in vulnerable tissues[72]. In the case of excess, Zn supplies can provoke gastric dysfunction, dizziness, nausea and a vulnerability of the immune system. In France, the recommended dose is 10 mg/d, the no adverse effect level has been set at 30 mg/d and the increase in needs has been estimated to be 0·24 mg/1000 kJ (1 mg/1000 kcal) over sedentary energy expenditure. So, there is no benefit of high amounts of Zn supplementation. Performance is correlated with the plasma Zn concentration only in the case of Zn deficit. A high daily ingestion of Zn decreases the absorption of Cu and decreases the Cu status which, in turn, decreases the Cu-Zn-SOD enzyme activity. The major consequence associated with the long-term ingestion of moderately high amounts of Zn is the generation of a secondary Cu deficiency, which has been noticed with individuals having a chronic intake of Zn supplements as low as 50 mg/d[73].

Lack of evidence for increased needs for Zn with physical exercise does not lead us to propose a supplementary intake of up to 0·24 mg/1000 kJ (1 mg/1000 kcal) of energy expenditure which can be provided by a diversified diet.

Copper

Among the many functions of Cu, focus will be made here on its antioxidant function via the antioxidant activity of Cu-Zn-SOD. Cu is carried by ceruloplasmin – a non-enzymic antioxidant – but 60% of Cu is bound to erythrocyte Cu-Zn-SOD. A deficit in Cu, when combined with exercise, leads to an alteration of the basal laminae of myocardial cells and to a decrease in the oxidative capacity of the soleus muscle in rats[74].

Losses of Cu through urine and sweat increase with exercise independently of the training level[67]. Losses are lower when Cu intakes are low, preserving thus a minimal Cu bioavailability as well as the Cu-Zn-SOD activity. To date, no study has shown evidence of the values of plasma Cu concentrations below the normal rate in physically active subjects even in cases of high sweat loss or high energy expenditure. Water and energy supplies necessary for the compensation of these losses allow the maintenance of cupric balance. An average Cu intake of 1·96 mg/d is found in highly trained subjects (energy expenditure $>16\,740$ kJ (4000 kcal)/d)[68].

Plasma Cu concentrations increase in response to exercise and return to initial values within a few days[75]. Thus, basal concentrations of plasma Cu could be mainly due to previous exercise. The decrease in erythrocyte Cu concentrations in response to exercise[75] suggests a mobilisation of Cu in response to exercise and could explain why plasma Cu concentrations are sometimes higher in athletes as compared with less active subjects. In fact, plasma Cu concentrations can increase during the training season, with a peak in concentrations after the competition period. Moreover, the activity of the erythrocyte Cu-Zn-SOD, one marker of Cu status, can vary with training loads[76]. In skeletal muscle, Cu-Zn-SOD activity is correlated with maximal O_2 uptake values[77]. Athletes who most risk Cu deficit are those who take high-Fe or -Zn supplement doses and those with high-glycaemic index carbohydrate ingestion because of the antagonistic action of those compounds on Cu intestinal absorption[73,78].

Selenium

The essential trace mineral Se as required for the functionality of selenoproteins is of fundamental importance to human health[79]. Selenocysteine is incorporated in at least twenty-five specific, genetically determined human selenoproteins, many of which have only recently been discovered[80]. Among well-known selenoproteins, glutathione peroxidases (GSH-Px), thioredoxin reductase and methionine sulfoxide reductase B are involved in the antioxidant defences.

In cases of Se depletion, Se is taken from muscle, carried and possibly deposited in tissues with a higher metabolic priority for Se such as the testes, brain and endocrine organs[81,82]. GSH-Px (isoform 1) protein concentration and activity are depleted in several tissues[82]. Se depletion leads to a morphological myocardial myocyte alteration, as well as to a disorganisation of the mitochondrial membrane. Moreover, the combined depletion of both Se and glutathione induces necrosis of both kidney and liver tissues[83]. Thiol groups contained in enzymes decrease with Se-deficient mice when subjected to an intensive training[84].

The bioavailability of Se seems to adapt when Se intakes are moderatly low or high. Faecal and urinary excretions decrease when the diet is poor in Se. Inversely, a readjustement in Se excretion is also observed along with an increase in urinary and faecal excretion when Se intakes are high[81]. This regulation of excretion does not occur when intakes are above or below a certain threshold[81].

Plasma Se concentrations of 1·15 μmol/l are known to maximise erythrocyte GSH-Px activity, which is used as a functional marker of Se status[79]. Some athletes exhibited plasma Se concentrations below this concentration[68]. However, most of highly and well-trained athletes generally adopt a high-Se diet spontaneously, which allows them to reach a high Se status[68]. The biological efficiency of Se in humans depends on the food source. It is therefore legitimate to question the nutritional Se density of athletes' diet but also to question the quality of the dietary sources. In healthy subjects, 39 % of Se is linked to plasma GSH-Px

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(isoform 3), 53 % to selenoprotein P and 9 % to albumin. Selenomethionine is mainly found in albumin. This Se distribution consequently varies according to the quality and to the quantity of dietary intakes[85]. Most of the Se in vegetables is in the form of selenomethionine whereas selenocysteine, which is used for selenoprotein synthesis, is mainly of animal food origin[79]. Se is also present in the form of selenite and selenate in food, both of which are also used for selenoprotein synthesis. As the percentage of plant protein supplies is higher in well-trained athletes in comparison with less trained athletes[68], Se supply coming from foods – of plant origin – rich in carbohydrates will be higher in the form of selenomethionine. The selenomethionine can be incorporated randomly in proteins instead of methionine. The part of non-metabolised selenomethionine is incorporated into tissues undergoing high protein turnover such as skeletal muscle and erythrocytes. Training introduces additional factors which induce an endogenous adaptive response of GSH-Px activity, probably mostly at post-translational level[44,86]. Factors such as ageing, sex or hypoxia exposure are also known to increase erythrocyte and/or plasma GSH-Px activity[44,87,88]. Therefore, the dose–response association between plasma Se concentrations and the erythrocyte GSH-Px activity is not always evidenced in athletes[44,68]. This raises the question of the validity of circulating GSH-Px activity as a Se status indicator in a training population.

With top-level athletes, Se supplementation with daily doses taken of 50 or 100 μg at once or in several intakes decreases lipid peroxidation and increases non-protein thiol groups[86]. However, others reported no effect of a daily supplementation (180 μg selenomethionine) during a 10-week training period with subjects originally less active on oxidative stress markers and on erythrocyte and muscle GSH-Px activities[89].

The increase in Se requirements with physical exercise is probably not linear[68]. The effect of the form of Se ingested (selenomethionine, selenocysteine, selenite, selenate) should be investigated as well as the effect of Se on other selenoproteins' functions. Data relating to the long-term effects of Se complements and supplements are lacking. A safety dose of 150 μg Se/d has nevertheless been suggested in France.

Is antioxidant micronutrient supplementation to be encouraged or discouraged?

Paradoxically, individuals who take supplements are generally those with the healthiest quality of life (non-smokers, with well-controlled physical activity programmes, etc) and whose fruit and vegetable consumption is high. Moreover, athletes who practise long-lasting activities often eat fortified foods. One can suppose that some athletes ingest more than they need. An excess of one antioxidant through supplementation could fail to provide a full antioxidant protection. It could also reduce the adaptive effect of the endogenous antioxidant system induced by physical training by minimising the effects of ROS themselves. These last play a major role in the signalling mechanisms that trigger the expression of antioxidant enzymes[70]. High doses of antioxidant supplementation have been shown several times to delay the exercise-induced stress protein increase and therefore the muscle homeostasis return. It seems that maintaining a normal antioxidant status through dietary intakes is essential in the adaptation process.

Exercise-induced oxidative damage can be alleviated by endogenous antioxidant adaptation. Antioxidant intakes determine the quality of the endogenous antioxidant system response[19]. The latter is dependent on training habits. In stable physiological conditions and with a reasoned choice of training loads, endogenous antioxidant system adaptation is proportionnal to training load. However, below and above a stimulation threshold triggered by a given load, the relationship between training loads and endogenous adaptation is not verified[76,90]. An interdependence of antioxidant intakes and the effects of training on the endogenous antioxidant system has been observed in reference to a training-load modulation (increase/decrease)[7,76]. This supposes that the antioxidants required during a period of increased or reduced training stress differ from those required during a 'normal' training period. Before a major competition, a training-load modulation (overloading/tapering), with initially well-adapted subjects, is a common strategy to reach a performance peak when the competition comes. Overloading is known to provoke a transitory or prolonged lack of physiological and/or biochemical adaptations. Endogenous antioxidant systems are not able to counteract the excess of ROS produced and oxidative stress is generated[90]. The maintenance of a normal nutritional status is determinant. If the effects of a supplementation during overtraining are mostly evident in the lessening of muscle damage, it is noteworthy that the supplied doses can be provided by a diversified and well-balanced diet[7]. The effects of antioxidant supplements are mainly visible when subjects are initially deficient in antioxidants or when the food ingested is limited in the given antioxidants[91,92]. Such a deficit is also visible in some subjects during the overloaded period of training. Available data still do not allow us to define the optimal antioxidant intakes that would protect overloaded or, even more so, overtrained subjects.

The intricate relationship between exogenous antioxidant intakes and training effects requires the linking of the interpretation of biological data to each individual case. In fact, the specificity of the adaptive response is the function of individual factors such as the training period, the type of physical activity performed, the training level, the age of the subjects, the environmental conditions and the infinite interindividual differences. In specific, identified cases, an antioxidant complex could help athletes with initially low antioxidant intakes to maintain their antioxidant status during an overloaded period of training.

Do data have a biological significance?

Beyond the limited relevance of the numerous markers, the statistical significance of changes in a result does not obviously mean that it has a biological significance. The variations seen during experimentation are transitory when exposure to stress is transitory. The reading of experimental results allows us only to guess the middle- or long-term effects of these complements or supplements.

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The plasma concentration of fat-soluble micronutrients such as α-tocopherol and carotenoids may depend on the expression level of gene coding for proteins strongly involved in the metabolism of lipids such as those activated by peroxisome proliferated activated receptors. The latter's level of expression, with regards particularly to isoform β/δ, varies with physical exercise[93]. Nucleotide polymorphisms on these genes may, on the other hand, provide an answer to the strong inter-individual variations of plasma α-tocopherol and carotenoid concentrations[6,44]. To this effect, a recent study showed that the presence of single nucleotide polymorphisms on the gene of SR-BI strongly influenced the concentrations of α-tocopherol and β-carotene[25]. However, as of date, the effect of physical exercise on the level of expression of this receptor is not yet known.

The clinician can rely on the RDA for the general and the specific population. He also has to pay particular attention to subjects with an atypical profile and keep an individualised approach.

The choice of markers depends on the function affected by the exercise and/or by the deficit of the nutrient intake (for example GSH-Px activity for Se status). With exercise, the biological data can only vary in a transitory manner because of a transfer from one compartment to another. The continuous evolution and increase in available markers would further help both researchers and clinicians to evaluate more precisely nutritional status.

The quality of the food composition table for trace elements, carotenoids and flavonoids is still considered to be poor. Moreover, quantities of nutrients within food vary among countries and studies are not always comparable. Due to the variability of trace elements in soils, especially in Se, and to the consequent variability of their content in the identical food, conventional methods to estimate Se intakes are not always adequate. Food diaries or questionnaires are often validated for a sedentary population and consequently need to be adapted and validated in a population of athletes. In fact, a limit of these questionnaires could be the underestimation of food intake by athletes due to large portion sizes. Another limit could be linked to the reporting of portion sizes by some subjects, mostly women, all the more in sports which have an aesthetic component. Thus, the dietary approach needs to be completed by a biological assessment. Considering the limits of the reliability of biological assessments and dietary surveys, mainly in athletic populations, only a full nutritional assessment including both a dietary survey and a biological assessment can lead to suggest to athletes, in particular conditions, complements to compensate for deficits. In cases in which the intakes are below the recommended intake level, the supplementation can become a 'complementation'. An enriched diet, with the same aim, could have similar effects.

Conclusion

Athletes are seeking methods to improve performance. Among those methods is the nutritional strategy. Low intakes can alter performance, as adequate intakes play a protective role. In this case, the dietary nutritional addition is only done with the goal of reaching an appropriate nutritional status and to restore biochemical and physiological functions using specifically chosen biomarkers. The long-term risks of deficiency, as well as excessive intakes of antioxidant micronutrients, are still unknown because they are still poorly investigated in athletes. A recent meta-analysis showed that antioxidant supplements, with the evoked exception of Se, may increase all-cause mortality[94]. The numerous studies carried out on antioxidant supplementation encourage caution as long as longitudinal studies in athletes do not allow the discarding of the hypothesis of antioxidant supplementation-linked risk.

High-training-stress periods are key periods for nutritional awareness. In most cases, the nutritional risk of deficit can be reduced by a rational application of recommended training loads and requirement can be satisfied by a well-balanced diet.

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