Free Radical Research, July 2007; 41(7): 829–838

informa
healthcare

The intervention of antioxidant therapy on platelet adhesion and immunomodulation in experimental physical stress

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Accepted by Professor A. Azzi

(Received 20 October 2006; in revised form 24 April 2007)

Abstract
During effort overstress the reactive oxygen species act chiefly on unsaturated lipids, inducing the formation of certain peroxidation products. We have investigated malondialdehide (MDA), platelet adhesion index, and immunological activation parameters during effort overstress and administration of vitamins E and C. Biochemical measurements were performed on erythrocytes and heart homogenate. In the vitamin E supplemented group, the platelet adhesion index was constantly correlated with the MDA level ($p < 0.001$). There is a protecting effect concerning the oxidative stress in animals pretreated with vitamin E and C, which is expressed through the diminution of the MDA quantity both in the erythrocyte and in the heart. The physical effort required by swimming led to a decrease in the NBT test values and in the activity of the serum complement. The steady administration of vitamin E in the effort overstress, due to its antioxidant properties, causes the progressive decrease in peroxidation and platelet adhesion.

Keywords: *Antioxidant vitamins, immunomodulation, platelet adhesion, physical stress*

Introduction

The influence of physical stress on immunity is an important issue for people involved in physical activity at all levels, from reactional jogging to top-level competition [1,2]. Many investigators have reported that moderate exercise appears to stimulate the immune system and to increase the immune response and that excessive training causes immune deficiency [3]. Coleman and Rager showed higher splenic proliferative responses in exercised rats compared to sedentary subjects [4].

Immune function is also affected by various stresses; thus, restraint stress decreased phagocytosis by neutrophils [5] and macrophages, diminished natural killer activity [6], radical scavenger activity and plaque-forming cell response, and changed the populations of T cells [7]. During effort overstress, due to the high oxygen consumption, the reactive oxygen species act chiefly on unsaturated lipids, belonging to the membrane, but also on other molecules in the cells, inducing the formation of certain peroxidation products, which generate malondialdehide (MDA). Free radical generation may lead to or result from platelet activation, suggesting that oxidative stress and platelet activation may be closely related as contributing factors to the evolution of atherosclerosis [8].

The protective, nutritional antioxidant function of vitamin E is also achieved and enhanced by other antioxidants, such as vitamin C, beta-carotene, glutathione (L-cysteine), and mineral selenium.

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ISSN 1071-5762 print/ISSN 1029-2470 online
DOI: 10.1080/10715760701416434

Vitamins E [9–11] and C [12] are endogenous scavengers of the oxygen free radicals. It is known that oxidants are involved in the development and clinical expression of coronary heart diseases and that antioxidants may contribute to disease resistance. Although the increased antioxidant intake generally involves increased consumption of antioxidant-rich foods, α-tocopherol intake levels are important and can be achieved only through supplementation.

Our investigation focused on the effect of physical stress on the immune response and on the role of the antioxidant vitamins C and E in immunomodulation. The aim of the present study was to evaluate platelet activity, by using platelet adhesion index, MDA and immunological activation parameters in experimental effort overstress, and by determining the physiopathological inter-relations regarding the role of antioxidant vitamins E and C in the mechanisms involved in the changes of oxidative stress, procoagulant status and immunomodulation. Understanding the specific mechanisms by which vitamins E and C exert their protective effects will help elucidate their potential roles in effort overstress and may ultimately lead to the development of successful preventive or therapeutic regimens.

Materials and methods

The experimental study fulfills all the requirements of the guide regarding the use of laboratory animals and biological preparations issued by the International Society of Pain Study (IASP) and the European Council Committee (86/609/EEC). Also, the study was evaluated and accepted by the committee of professional ethics of Gr. T. Popa University of Medicine and Pharmacy from Iasi (9803/12.09.2006).

We used DL-α-tocopherol, because its incorporation into the vascular wall prevents the endothelial dysfunction. Theoretically if we used the same dose in oral and intramuscular administration we should obtain the same results. We favored intramuscular (i.m) administration, because we can obtain faster and more reliable results, as the whole dose benefits from a safer administration and a better absorption under this form, as compared to oral administration, which may cause injuries in case of daily administration by tube-feeding. Moreover, vitamin E is liposoluble and should be administered separately from vitamin C, which is water-soluble.

In our experiment we used 210 male white Wistar rats (they weigh 150–200 g), divided into 7 groups, namely: group 1, the control group; group 2, animals to which vitamin E was administered i.m. in doses of 4 mg/100 g of body weight at 48 h intervals for one month; group 3, animals to which vitamin C was administered i.m. in doses of 30 mg/100 g of body weight at 48 h intervals for one month; group 4, daily treated animals, alternatively with vitamins C and E at above mentioned intervals and doses; group 5, animals which performed daily physical exercises by swimming for 10 min for one month; group 6, pretreated animals with vitamins C and E for 2 weeks and which were subjected daily to physical effort by swimming for 2 weeks; group 7, animals to which vitamins C and E were administered for one month, at the mentioned intervals and doses, concomitantly with physical effort.

Before being slaughtered, the rats were anesthetized with conditioned ketamin in vials of 5 ml (50 mg/ml). About 2.5 ml ketamin were dissolved in 10 ml physiological serum and administered to rats in doses of 1 ml/100 g intraperitoneally. Blood samples were drawn directly from the ventricle by means of a syringe, then the heart was perfused with physiological serum for some minutes. The heart homogenate was prepared 1 g:10 in solution of NaCl 0.9 g%. To obtain red cells, blood was centrifuged at 3500 rpm, after which the sediment was washed with NaCl 0.9 g% until the full removal of the hemoglobin resulted from the injured red cells.

Biochemical measurements were made on erythrocytes and heart homogenate.

The MDA concentration

The index of lipid peroxidation, was determined by the Ohkawa method using the tiobarbituric acid [13]. The MDA concentration was expressed in nM/mg protein or g Hb.

Leukocytic formula

The differential count, expressed as the percentage of each type of cell, was related to the total leukocyte count.

NBT test

The Nitroblue tetrazolium (NBT) test points out the metabolic changes during the ingestion processes, quantitatively correlated with the phagocytosis and identified by the NBT reduction. NBT solution was obtained from 1 mg powder NBT diluted in 0.5 ml phosphate buffer (pH = 7.2) and 0.5 ml physiological serum (final pH of the solution was 7.2). The solution was warmed at 37°C. Smears were made then they were colored using the Giemsa or Gram methods and afterwards they were examined microscopically. The percentage of the NBT positive neutrophils was recorded for further use.

Determining the activity of the serum complement

The complement hemolyses the sensitive erythrocytes (erythrocytes "coated" by specific antibodies).

The 50% (CH_{50}) hemolysis was used because it renders more precision to the dosage of the serum complement than the 100% hemolysis. The serum dilutions were obtained with veronal buffer (pH = 7.3) diluted, in its turn, with 1/5 physiological serum. The reaction witness was H_{50} (50% hemolysis) = 0.5 ml hemolytic system + 3.5 ml distilled water. Each sample was read with the spectrophotometer, comparing it with H_{50} and the values of the serum complement were expressed in CH_{50} units, observing the dilution from the mixture which produced 50% hemolysis.

Platelet adhesion index

Platelet adhesion was established as platelet adhesion index (AI) by using Rovatti's method. Platelet count was made before and after glass adherence by using the visual method in EDTA solution. AI = platelet count without glass adherence/platelet count after glass adherence.

Statistics interpretation

Data were analyzed with One-Way ANOVA test for variances analysis with a SPSS ver. 13.0. computer program that allows the comparison of the mean values for three or more groups defined by group variable. This method enables one to extend the analysis performed by *t*-test applicable to two mean values (we compared two by two). Single-factor ANOVA test outcome interpretation tackles two tests:—*test of homogeneity of variances*–which is necessary in order to determine which test is adequate for mean value comparison. The null hypothesis is rejected if the Sig. value (significance thershold) is lower than 0.05 (5%), meaning that all variances are not equal; *ANOVA test*–the null hypothesis is rejected if the Sig. value is lower than 0.05 (5%), meaning that at least two mean values, calculated at the subpopulation level, are different from each other.

All the data are shown as mean value ± standard error of the mean (SEM). Statistical analysis was performed with the paired or unpaired *t*-test, and *P*-values inferior to 0.05 were considered statistically significant.

Results

The granulocytes and especially the polymorphonuclear neutrophiles (PMN) play an important role regarding the destruction of microorganisms, being involved into acute and chronic inflammatory processes. They show a high reception potential for the chemotactical stimuli, a high mobility and very active phagocytic functions.

After the "fagocytation" of the "aggressors", their destruction by the PMN depends on the activation of the granular enzymes and of the NADPH oxydase, followed by the release of the toxic molecules of oxygen. PMN carries out its ("killing") "cide functions" by means of two distinct mechanisms: one depending on the oxidative metabolism and the other independent of this metabolism.

Leukocytic formula

The performance of the Student-*t* test showed significant statistical differences between group 5 and groups 6 and 7 ($p < 0.001$). The variation in the PMN percentage was statistically significant in the groups treated with vitamin E plus vitamin C for a month (group 4) and in the animals of the groups 6 and 7 ($p < 0.001$).

Since Sig. < 0.001 (0.000), the null hypothesis was rejected, therefore, there were differences between the mean values of the groups (4 as compared to 6 and 7), however with a relatively low variation within the groups (*F*-value = 0.615–61.463%). (Figure 1)

As compared to group 1, the animals belonging to the groups treated with vitamin E (group 2) and the animals treated with vitamin E plus C for a month

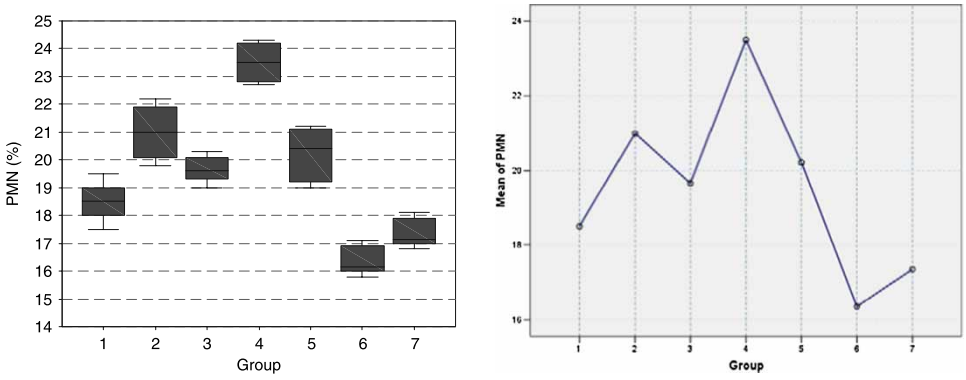

Figure 1. Leukocytic formula in rats: a. PMN (%). In comparison with the control group, the animals from group 2, group 3 and group 4, respectively, showed increases of the percentage of PMN in the leukocytic formula. The animals from groups 6 and 7 showed a decrease in these elements of the peripheric blood ($p < 0.01$). b. ANOVA test. Since Sig. < 0.001 (0.000), there are differences between the mean values of the groups (4 as compared to 6 and 7), however with a relatively low variation within the groups (*F*-value = 0.615–61.463%).

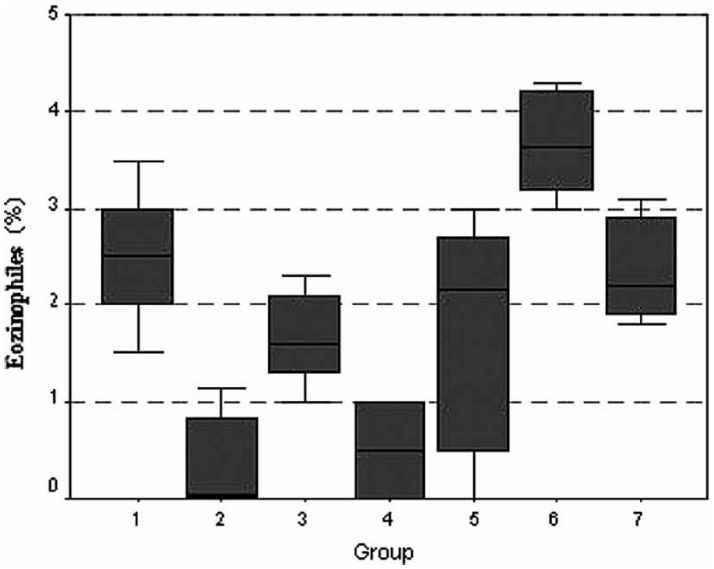

Figure 2. Leukocytic formula in rats. Eozinophiles (%). In comparison with the group 1, the animals belonging to the groups 2 and 4, showed from a statistical point of view, significant decreases of the percentage of the eosinophiles in the leukocytic formula ($p < 0.01$).

(group 4), showed, from a statistical point of view, significant decreases of the percentage of the eosinophiles in the leukocytic formula—$p < 0.01$. (Figure 2)

Phagocythosis potential of the PMN of peripheral blood (NBT test)

As the Levene homogeneity test (test of homogeneity of variances), Sig. < 0.05, showed that the variances were not equal, the ANOVA test could not be applied to all the seven groups, and we had to limit ourselves to performing the comparison by the Student-*t* test (two by two).

As compared to the animals belonging to group 1, the animals of groups 2 and 3 as well as those of group 4 showed increases of the NBT test values [14], while animals belonging to groups 6 and 7 showed decreases of this parameter in comparison with the animals belonging to group 5. The performance of the Student-*t* test showed significant statistical differences between group 5 and groups 6 and 7 ($p < 0.001$; Figure 3).

The serum complement

Since on the homogeneity test Sig. < 0.05, the variances were not equal and consequently the ANOVA test could not be applied to all the seven groups (the standard deviations in groups 2 and 6 were high), which allowed us to perform only the comparison by means of the Student-*t* test (two by two).

As compared to the control group, the animals of groups 5, 6 and 7 showed an activation of the serum complement (Figure 4), the values not being significant from the statistical point of view ($p > 0.05$). In the experimental model that we used, the physical effort of swimming led to the cancellation of the PMN activity (decrease of the NBT test values) and of the activity of the serum complement.

The platelet adhesion index and MDA

Vitamin E may modulate platelet adherence and aggregation and thus prevent progression of a fatty streak and cell proliferation to an advanced lesion. Decreased vitamin E concentrations are associated with increased aggregation, which is reversible by improving vitamin E status [15–16].

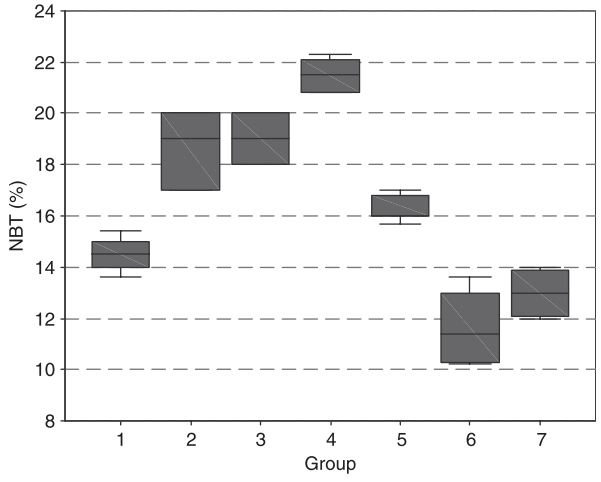

Figure 3. NBT test in rats (%). In comparison with the animals belonging to the group 1, the animals from groups 2, 3, and 4, as well as those from group 6 showed increases of the NBT test values, while animals belonging to the groups 5 and 7 showed decreases of this parameter ($p < 0.01$).

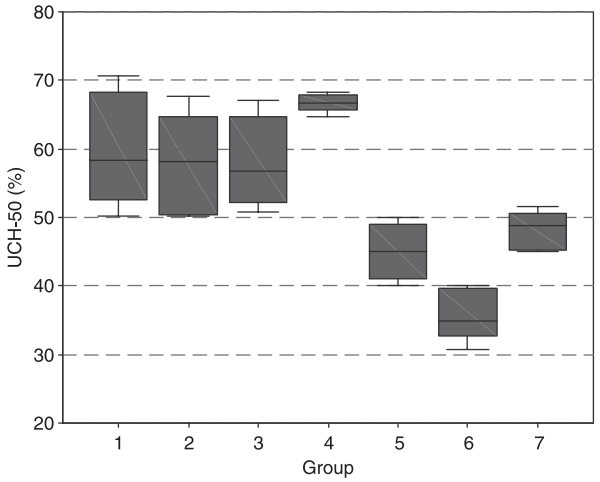

Figure 4. Serum complement in rats (U CH_{50}). Comparing with the control group, the animals under stress and treated or not treated with vitamins E plus C, showed an activation of the serum complement, the values not being significant from the statistical point of view ($p > 0.05$).

In the vitamin E supplemented group (group 2), the platelet adhesion index was constantly correlated with the MDA level and less correlated with other metabolic disorders.

For the platelet adhesion index, since Sig. < 0.001 (0.000), the null hypothesis was rejected, and, therefore, there were differences between the mean values of the groups (1 as compared to 2, 6 and 7), accompanied however by a very low variation within the groups (F-value $= 3450.533$; Figure 5). The performance of the Student-t test showed significant statistical differences between group 5 and groups 6 and 7 ($p < 0.001$).

Our study focused on MDA in the heart homogenate and in the erythrocytes because the studies on isoprostanes are still under debate. For the MDA in the erythrocyte, Sig. < 0.05, the variances were not equal, and therefore we could not apply the ANOVA test on all the groups. As a result, we had to exclude the outlier values of groups 1 and 4 (extreme value-0). After this exclusion (the missing option of the program), the null hypothesis was rejected, and, therefore, ANOVA was taken into consideration. Since Sig. < 0.001 (0.000), the null hypothesis was rejected, and therefore, there were differences between the mean values of the groups (5, 6 and 7) and a high variation within the groups (F-value $= 0.45–44.7863\%$).

The MDA, an unspecific, but relevant parameter of the lipid peroxidation process is present in high concentration in the erythrocyte, but it is absent in the heart of the animals subject to swimming-induced experimental stress. Pretreatment and treatment with antioxidant vitamins E and C reduced the MDA concentration in the heart to zero (the heart of the animals from the 5th group) (Figure 6).

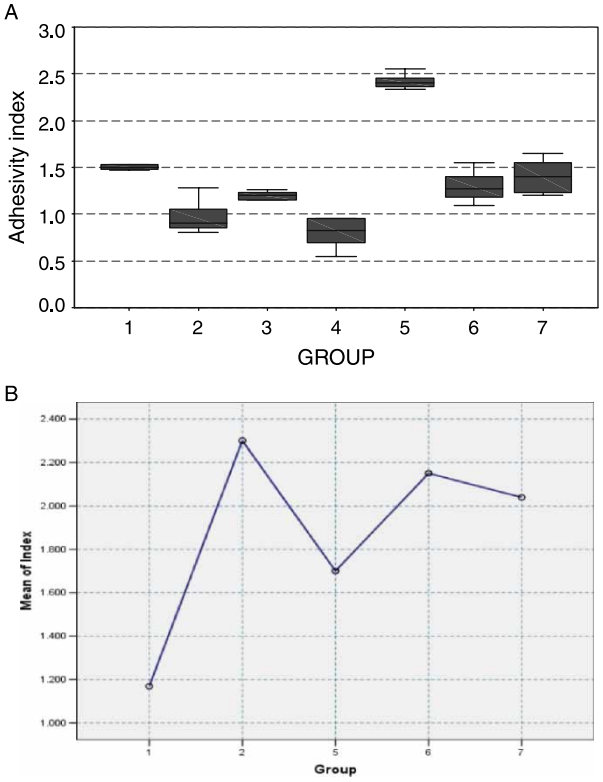

Figure 5. a. Absolute values of the platelet adhesion index. The adhesivity index was decreased on groups 4, 6, and 7 in comparison with group 1, and significantly increased on group 5 ($p < 0.001$). b. ANOVA test. Since Sig. < 0.001 (0.000), there are differences between the mean values of the groups (1 as compared to 2, 6 and 7), however with a very low variation within the groups (F-value $= 3450.533$).

For the MDA in the heart homogenate, Sig. < 0.05, the variances were not equal, and therefore the ANOVA test could not be applied to all the groups (the standard deviation in group 5 was zero). As a result, it was necessary to exclude the outlier value of group 5 (extreme value-0). After this exclusion (the missing option of the program), the null hypothesis was rejected, and therefore ANOVA was taken into consideration. Since Sig. < 0.001 (0.000), the null hypothesis was rejected, and ,therefore, there were differences between the mean values of the groups (5, 6 and 7) and a very low variation within the groups (F-value $= 276.777$).

Discussion

The changes of the lipid peroxides were pointed out through the determination of the MDA in the erythrocyte and in the heart homogenate, in rats subject to experimental stress. Malondialdehide concentration revealed a slightly prooxidative behavior of the E and C vitamins that explained the only partial recovery of enzymatic activity to normal values, as well as a moderate lipid peroxidation process. Both phenomena were better expressed in erythrocytes.

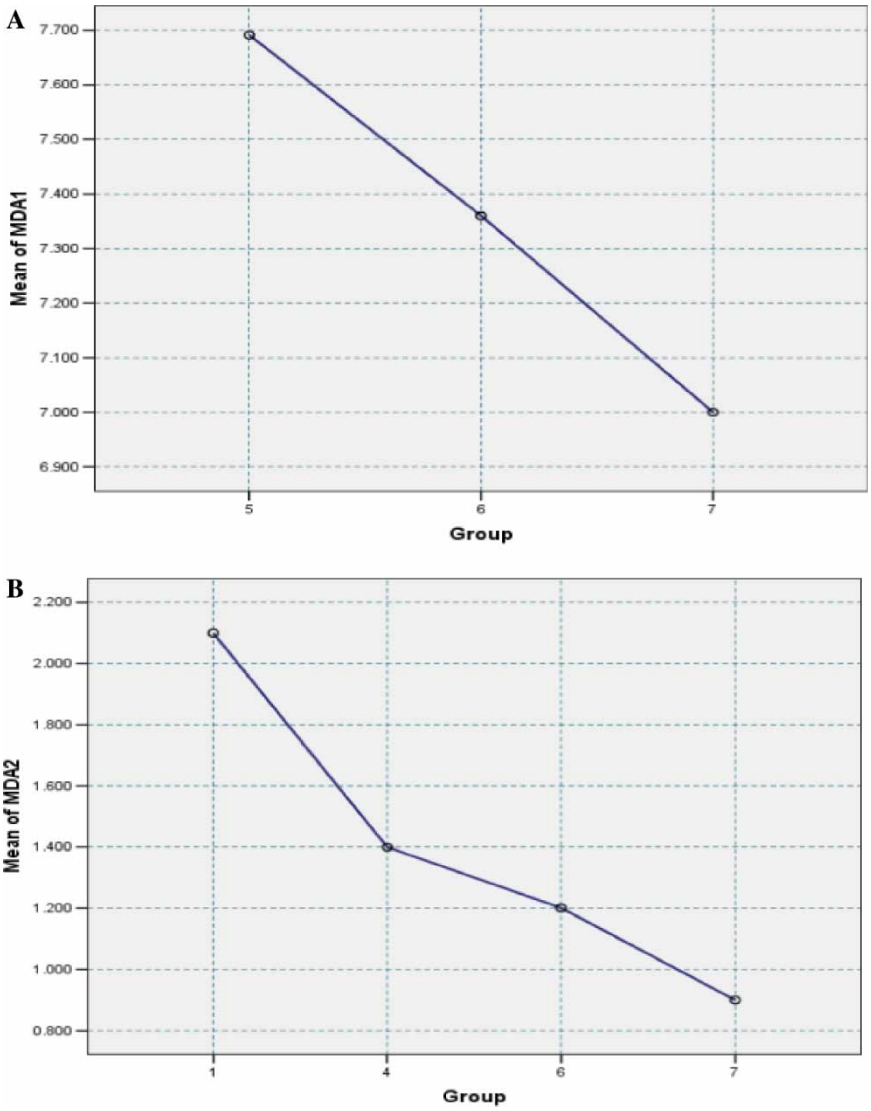

Figure 6. a. MDA in the erythrocyte (MDA1). Since Sig. < 0.001 (0.000), there are differences between the mean values of the groups (5, 6 and 7) and a high variation within the groups (F-value = 0.45–44.7863%). b. MDA in the heart homogenate (MDA2). Since Sig. < 0.001 (0.000), there are differences between the mean values of the groups (5, 6 and 7) and a very low variation within the groups (F-value = 276.777).

The MDA absence in the heart of the animals subject to experimental stress, through physical exercise, is explained through the reduction of its production or removal rate following the raise of tissue vascularization or of both. It is also possible that MDA is metabolized, under effort, in the old animals, up to CO_2. In our experiment, it is difficult to say whether the absence of MDA in the heart of the animals which were administered vitamins E and C for the entire period of the exercise is due to a *per se* action of the two vitamins or to the improvement of the above-mentioned ways to remove the MDA by the cell.

Our results point out a protecting effect concerning the oxidative stress produced by effort overstress in animals pretreated with vitamin E, which is expressed through the diminution of the MDA quantity both in erythrocyte and in the heart. In the absence of a certain oxidative stress, vitamin E administration was associated with a high MDA level, a situation which suggests that vitamin E, as a radical oxygen species, may produce by itself a pro-oxidative influence under certain metabolic conditions, when there is no preexistant oxidative stress.

Bouts of intense exercise are associated with increases in lipid peroxidation, generating MDA and $F_{2\alpha}$-isoprostanes, and the release of muscle enzymes like lactate dehydrogenase and creatine kinase. $F_{2\alpha}$-isoprostanes derived from arachidonic acid have been associated with oxidant injury and appear to be a much more specific biomarker of lipid peroxidation than MDA [17,18]. In contrast, Mori et al. [19] examined for 8 weeks patients with type 2 diabetes, who underwent moderate- or low- intensity exercise training and found that neither of these training regimens affected urinary isoprostane excretion. Wang et al. [20] trained (10 weeks of treadmill exercise) with

untrained rats and found that the trained group had lower urinary excretion rates of isoprostanes.

For this reason, we considered that the MDA in the heart homogenate and in the erythrocytes may be the proof of the radical endogenous oxidative stress, the MDA being the end result of lipid peroxidation.

Deficiencies in tocopherol and riboflavin reduce cell numbers in the lymphoid tissues of the animals used in experiments and produce functional abnormalities in the cell mediated immune response. Ortega et al. [21] reported a significantly higher chemotaxis, ingestion and microbicide potential of neutrophils in elite sports-women than among sedentary young women. Neutrophils also play a pathogenic role through the same cytotoxic mechanisms in non-infectious inflammatory diseases such as arteriosclerosis, myocardial infarction, asthma and adult respiratory distress syndrome. Studies on exercise show a relation between exercise intensity and the neutrophil functions of elite athletes [22], sedentary people [23,24] and animals used in experiments [25].

Some vitamins like A, C, D and E possess the ability to unspecifically activate the cytotoxic functions of the Tc lymphocytes or of the NK cells, and to normalize the cellular cooperating mechanisms [26–29]. Ascorbic acid and tocopherols are important not only for limiting tissue damage but also in preventing increased cytokine production. α-Tocopherol appears unique in regulating phosphorylation cascades. Such a role may be important in heart disease where cell adhesion, proliferation, and oxidant production may all be modified through vitamin E-sensitive pathways.

Concerning the platelet adhesion index, it is supposed that, given the oxidative stress triggered by effort overstress, although the proaggregant potential is high, which is pointed out by the increase of the primary hemostasis indexes, platelet metabolism, secretion and aggregation diminish with physical effort. We may suggest that the procoagulant status accompanying the oxidative stress of the experimental physical stress is due not to the thrombocyte, but to the decrease of the anticoagulant attributes of the endothelium.

Vascular thrombogenicity is induced by progressive LDL oxidation and alterations of the antioxidant/oxidant balance of the LDL particle in favor of the antioxidant tone are protective against the thrombotic response triggered by oxidative stress [30–34]. Vitamin E has been shown to inhibit monocyte tissue factor (TF) expression *ex vivo* and clinical studies indicate that vitamin E supplementation is able to significantly reduce monocyte TF expression, a marker of thrombin generation *in vivo* [35]. The most potent antioxidant form of vitamin E, α-Tocopherol, is bound mainly to lipoproteins in plasma; its incorporation into the vascular wall prevents the endothelial dysfunction at an early stage of atherosclerosis [36].

The increase of oxidative stress, not related to blood pressure values, is accompanied by a reduction in the most important antioxidant mechanisms and by the accumulation of ROS byproducts, not only from lipid peroxidation, but also from oxidized genomic and mitochondrial DNA [37]. The impact of the released ROS products from the peripheral mononuclear cells may contribute to the endothelial dysfunction and to the organ damage present in effort overstress.

α-Tocopherol inhibits smooth muscle cell proliferation, decreases protein kinase C (PKC) activity, and increases phosphoprotein phosphatase 2A activity [38]. α-Tocopherol effects on PKC inhibition have also been reported in human platelets and monocytes [39]. The mechanism of PKC inhibition by α-tocopherol may be attributable in part to its attenuation of the generation of membrane-derived diacylglycerol, a lipid that activates PKC translocation and activity [40]. The inhibition of PKC activity is not due directly to the antioxidant potential of α-tocopherol, but requires the integration of α-tocopherol into a membrane structure, and is likely to take place due to the direct interaction between α-tocopherol and PKC in the cell membrane. For this reason we suggested that the procoagulant status accompanying the oxidative stress of the effort overstress is due to the decrease of the anticoagulant attributes of the endothelium. Increase in antioxidant defenses might not be physiologically proportionate to the needs created by the increase in prooxidant events and this might affect the requirement for dietary antioxidants such as vitamins E and C.

Vitamin E enrichment of endothelial cells down-regulates the expression of intercellular cell adhesion protein and vascular cell adhesion molecule-1, thereby reducing the oxidized LDL-induced adhesion of white cells to the endothelium [41]. Vitamin E up-regulates the activities of cytosolic phospholipase A2 [42] and cyclooxygenase [43]. The enhanced activity of these two rate-limiting enzymes in the arachidonic acid cascade provides a mechanism allowing us to observe that vitamin E dose-dependently enhances release of prostacyclin, a potent vasodilator and inhibitor of platelet aggregation. Thus, vitamin E has been shown to inhibit phospholipase A_2 activity, alter phospholipid metabolism, reduce platelet release of thromboxane A_2, and inhibit platelet aggregation. Vitamin E is one of the few nutrients for which higher than recommended amounts have been shown to enhance the immune response and might be needed to maintain the optimum immune response [44].

Vitamin E stimulated the phagocytosis potential of the PMN of peripheral blood stimulating, in particular, the PMN independent oxydative mechanism. Immunological activation of endothelial cells is accompanied by the appearance at the cell surface of increased numbers of the leucocyte adhesion molecules E-selectin, a phenomenon which is also specific

for the endothelium. Therefore, injury to the endothelium by oxidative stress could be a mechanism or a major risk factor for the atherosclerotic process. Repeated physical effort produces a lowering of the total antioxidants, reducing thus the capacity to dissociate free radicals. It seems that, in these circumstances, the decrease of antioxidant mechanisms is the cause and not the effect of oxidative stress. In ultra-endurance events, oxidative stress is high and antioxidant levels are compromised. Combinations of antioxidants may be of particular benefit because of the possible synergistic interaction between vitamin C and α- tocopherol. Vitamin C preserves vitamin E in LDL during oxidative stress and protects lipoproteins by coupling the glutathione redox system to vitamin E. It can actually produce a synergistic effect where the combined effect is greater than the sum of the individual antioxidants.

α-Tocopherol, the most active form of vitamin E, is the predominant lipophilic antioxidant in LDL. In addition, α-tocopherol supplementation has other beneficial effects, such as suppression of a potentially atherogenic cytokine, IL-1β and inhibition of a crucial event in atherogenesis, monocyte-endothelial cell adhesion [45]. The release of IL-1β and monocyte-endothelial cell adhesion seem to be regulated via other mechanisms such as activation of transcription factors like NFkb. The inhibition of IL-1β release and monocyte-endothelial cell adhesion by α-tocopherol is possibly due to its antioxidant effect and moderation of the intracellular oxidative stress [46].

γ-Tocopherol is somewhat less potent in donating electrons than is α-tocopherol and thus, is a slightly less powerful antioxidant [47,48]. As such α-tocopherol is generally considered to be more potent than is γ-tocopherol as a chain-breaking antioxidant for inhibiting lipid peroxidation [49].

The imbalance between pro- and anti-oxidants in effort overstress may enhance platelet adhesion via arachidonic acid metabolism and endoperoxides formation. Increased platelet adhesion index and high levels of soluble E-selectin predict an increased risk of coronary artery disease or peripheral vascular disease.

One can suggest that E and C antioxidant vitamins influence the phagocytic activity of the PMN, stimulating, in particular, the PMN independent oxidative mechanism. The activated PMN show the receptors for C3b of the complement so that the animals submitted to stress and concomitantly treated with vitamins E plus C show a consumption of serum complement (activation of serum complement).

These results suggest that physical stress has the effect of increasing superoxide production in the case of low phagocytic activity and decreasing superoxide production in the case of high phagocytic activity, showing a disturbed functional balance. Impairment of the immune response has been associated with repeated or prolonged stress. Especially in severely stressed conditions, immune reactivity could be downregulated to a point that provides inadequate protection to infections or causes autoimmunity.

Positive correlations show the existence of good efficiency between phagocytic activity and superoxide production of neutrophils. Another hypothesis resulting from the above mentioned affirmations might be that of use of the activating immunomodulators of the newborn and/or adaptive defense, besides substances that activate the antioxidating systems of the organism or preserve them. Vitamin C and E, by their antioxidizing effect could function as efficient modulators in immunodepressions, in ischaemic, tissue degenerative and inflammatory processes.

However, when an athlete competes in longer events, antioxidants may become more effective and, therefore, the athlete should pay closer attention to his overall eating and supplementation program.

Conclusions

Steady administration of vitamin E in effort overstress, due to its antioxidant properties, causes the progressive decrease of lipid peroxidation and of platelet adhesiveness. Vitamins C and E stimulated the phagocytosis potential of the PMN of peripheral blood, but did not influence the serum complement. Preventive treatment with vitamin E reduces the expansion of the lipid peroxidation in the heart tissue and offers protection against the ischemia–reperfusion lesions through the decreasing of oxidative stress.

Under conditions of experimentally-induced oxidative stress, an antioxidants–prooxidants disequilibrium is caused; it depends on the tissue and on the time of administration of vitamins E and C. These vitamins with an antioxidant role may be used together with the immunomodulators in the conditions of a physical stress experiment. Vitamins C and E, through their antioxidant effect, may be efficient modulators in the ischaemic and tissue degenerative processes, as well as in the inflammatory processes.

The multiple interrelations of these results appear to reflect a diversity of factors including the antioxidants tested, the nature and timing of the exercise, the age of the subjects, and the methodology for assessing oxidative stress.

Future studies will aim at analysing the effect of α- or γ-tocopherol supplementation in association with ascorbic acid on immunomodulatory properties and will examine other markers of oxidative stress.

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