

Forum Review

The Immune System in the Oxidative Stress Conditions of Aging and Hypertension: Favorable Effects of Antioxidants and Physical Exercise

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ABSTRACT

Several studies have shown that both oxidative stress and inflammation are linked to the process of hypertension and that the immune system is also involved in this age-related process. More specifically, the oxygen stress related to immune system dysfunction seems to have a key role in senescence, in agreement with the oxidation/inflammation theory of aging. From a practical point of view, and according to our own research, the immune functions change in a similar fashion in hypertension and aging. As antioxidant diet supplementation decreases oxidative stress, it may be useful to treat hypertension and increase longevity. Probably, these favorable effects are mediated by an antioxidant-induced improvement of the immune function. The practice of moderate physical exercise shows similar favorable effects, and indeed our studies on exercising hypertensive women demonstrate an improved immune function, probably linked to raised levels of intracellular antioxidant defenses. The present review summarizes a selection of data related to the above from other authors as well as some findings from our own work. *Antioxid. Redox Signal.* 7, 1356–1366.

INTRODUCTION

OXIDATIVE STRESS seems to play a key role in the pathogenesis of hypertension and aging. Indeed, a great number of observations accumulated in the last decades suggest the presence of an oxidant–antioxidant imbalance in both the aging process and the hypertensive condition. Moreover, the oxidative and inflammatory processes are closely related, and the immune cells are an important source of both oxidant and proinflammatory compounds. For this reason, and because vulnerability to oxidative stress in aging and hypertension has been usually linked to an impairment of antioxidant defenses (43, 87), the antioxidant therapy has been used to prevent vascular alterations and improve the health of aged subjects (13, 46, 49). Antioxidants could exert these beneficial effects through an improvement of immune cell functions (10, 23, 46). Another method to increase the health of the aged is to

perform physical exercise (12), which could involve different mechanisms among which the improvement of the immune functions may be important. Physical exercise is also useful in the treatment of hypertension (27). However, the effect of moderate physical exercise on the immune functions of hypertensive subjects has been scarcely studied. Probably, moderate physical exercise improves the immune cell functions by increasing the antioxidant competence in these cells.

HYPERTENSION, OXIDATIVE STRESS, AND INFLAMMATION: THEIR RELATION TO THE IMMUNE SYSTEM

The complete mechanism of hypertension, a strong contributor to cardiovascular disease, has not been elucidated.

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However, there is growing evidence that oxidative stress may play a critical role in the pathogenesis of hypertension, as well as of other cardiovascular disorders such as atherosclerosis and myocardial infarction (78). An excessive production of reactive oxygen species (ROS), which is associated with inflammation and with a decrease in antioxidant defenses, leads to a condition of oxidative stress, which is a major contributing factor to the high morbidity and mortality rates associated with the above-mentioned disorders. It has already been proposed that hypertension is associated with a vascular inflammatory response (76), in agreement with the fact that untreated patients with mild to moderate arterial hypertension show elevated plasma levels of inflammatory mediators such as chemokines, adhesion molecules, and several proinflammatory cytokines (57). Moreover, the impairment of endothelial cell functions linked to hypertension is associated with an increased production of superoxide radical (3) and an overexpression of adhesion molecules (47) in the endothelium. An increase in the levels of oxidized glutathione (GSSG) and a decrease in the reduced form of that antioxidant (GSH) have also been shown in the blood of rats with hypertension (3) and in red blood cells of hypertensive patients (52, 79). A decrease of other antioxidant defenses, such as vitamin C, vitamin E, and β -carotene in plasma and superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in erythrocytes from hypertensive patients, has also been demonstrated (82). As the result of this oxidative stress, these patients show an increased lipid peroxidation in their plasma and erythrocytes (82). As an oxidative/inflammatory pathology, hypertension is related to an altered function of immune cells (76). Thus, the oxidative stress in immune cells from hypertensive patients is increased in comparison with that present in the normotensive controls (88), and the spontaneous form of hypertension in rats is accompanied by immune dysfunction and increased production of prooxidant/proinflammatory compounds by leukocytes (47, 76). In fact, dating back to over 20 years ago, it has been known that changes in the immune cells from hypertensive patients can contribute to or aggravate the hypertension-related vascular damage and are therefore pathogenically relevant. Accordingly, the increased T-lymphocyte reactivity against human arterial antigen is more common in patients with hypertension than in matched control subjects, and the serum levels of immunoglobulins G and M, as well as of autoantibodies, are also significantly higher in these patients (25). Moreover, the renal infiltration of immune cells and the interrelation between oxidative stress and the inflammatory response in which they are involved have been associated with the pathogenesis of hypertension (67). The circulating levels of several cytokines and their soluble receptors are also changed in hypertensive patients, with an increased secretion of proinflammatory cytokines, such as interleukin-1 β (IL-1 β) or tumor necrosis factor- α (TNF α), by peripheral blood monocytes from these patients (77). Another leukocyte parameter that increases with oxidative stress is the expression of intercellular adhesion molecules, which is higher in the lymphocytes from women with pregnancy-induced hypertension (86).

AGING: A CHRONIC OXIDATIVE STRESS. ROLE OF THE IMMUNE SYSTEM

Aging may be defined as a progressive, endogenous, and irreversible accumulation of adverse changes that increase vulnerability to disease and finally to death. Among the more than 300 theories proposed to explain the process of aging (44), the free-radical concept proposed by Harman (29) attracts a great deal of attention. This theory, which was further developed by Harman (30), Miquel (48, 50), and others (56, 71), postulates that aging is the consequence of accumulation of damage in biomolecules caused by the free radicals produced in our cells as a result of the necessary use of oxygen. As oxygen is mainly used in respiration to support the life-maintaining metabolic processes, the mitochondria, and more concretely their DNA (mtDNA), are probably the first target of that oxidation. As first pointed out by Miquel *et al.* (48, 50), it is in the postmitotic cells that cannot regenerate fully those organelles where the aging process starts. Moreover, the rate of mitochondrial oxygen radical generation, as well as the degree of membrane fatty acid unsaturation, and the oxidative damage to mtDNA are lower in the long-lived than in the short-lived species (56). Thus, the mitochondrial damage caused by free radicals results in a loss of bioenergetic competence that leads to aging and death of cells, and therefore of the organism (48, 50).

The aging process is very heterogeneous. Thus, there are different rates of physiological changes in the various systems of the organism and in the diverse members of a population of the same chronological age. This justifies the introduction of the concept of "biological age" or "functional age," which is very useful to assess the level of aging experienced by each individual, and therefore his life expectancy (4). Aging is associated with a great number of changes at all levels of biological organization, and there is a need to select parameters that are useful as biomarkers of aging. Presently, as the immune function is a marker of health and longevity (85) and a positive relation has been shown between a good function of T lymphocytes and natural killer (NK) cells and longevity, the immune parameters can be considered appropriate biomarkers of biological age. In fact, our group has proposed as biomarkers several immune parameters (7, 21, 22, 24). Thus, several immune functions of phagocytes (such as chemotaxis and ingestion of particles) and of lymphocytes (such as migration, proliferative response to mitogens, and IL-2 release), as well as NK cytotoxicity against tumoral cells, decrease with aging (7). However, other functions, very closely linked to oxidative stress, such as adherence of leukocytes to inert substrate and production of proinflammatory cytokines (namely, TNF α) are those that increase with age. All these changes with aging are similar in leukocytes from mice and humans (7). It is possible that nearly every component of the immune system undergoes striking age-associated restructuring, leading to changes that may include enhanced, as well as diminished, function. The above parameters, which we have standardized at different ages in mice and humans, have been confirmed as markers of biological age using a model of premature aging in mouse. These prematurely aging

mice (PAM) show the above-mentioned diverse functional parameters investigated (in phagocytes, lymphocytes, and NK cells) with values characteristic of chronologically older animals. Moreover, we have observed that these PAM show a significantly decreased life span (21).

The changes that occur with age in the function of the immune cells are due in great proportion to the "chronic oxidative stress" to which they are exposed in the course of time. In fact, recent studies of our group show that the production and release of oxidant and proinflammatory compounds, such as extracellular superoxide anion, TNF α , prostaglandin E₂ (PGE₂), GSSG, and the ratio GSSG/GSH (an index of the oxidative state), increase with age. By contrast, the level of antioxidant defenses, such as GSH, SOD, CAT, GPx, and glutathione reductase (GR), decrease in peritoneal leukocytes from mice. As a consequence of this oxidative stress, an increase in biomolecular damage, such as lipid peroxidation assessed as malondialdehyde (MDA), and DNA damage detected by the increase in 8-oxo-7,8-dihydro-2-deoxyguanosine (8oxodG), appears (11). These age-related changes are summarized in Table 1. Many of these changes, at the level of both function and oxidative stress parameters, appear also in leukocytes from peritoneum of mice suffering "acute oxidative stress" caused by an endotoxemia (83). Thus, we have observed in mice, in which an "endotoxic shock" is induced by injection of bacterial lipopolysaccharide (LPS; from *E. coli*), a 100% mortality at 30 h after the infection was provoked. The evolution induced by the functional and oxidative stress parameters in immune cells is similar in the relatively few hours of survival after LPS injection and in the ~24 months of life in control mice. An important question related to the above, as well as to the changes in immune cells in normal aging and its causes, is whether the changes linked to chronic oxidative stress are only one more of the results of the oxidative reactions that take place with the passage of time or whether they can be an important cause of the general age-related changes. We should remember that the immune cells, in order to fulfill their defensive function, show an inflammatory response,

producing factors, such as TNF α and ROS, which support the inflammation and oxidative process that allow the elimination of the antigen. As the oxidant and proinflammatory factors are increased with age, a new theory of aging, namely, the "inflammation theory," is being developed (Fig. 1). In fact, a transcription factor as ubiquitous as the nuclear factor- κ B (NF- κ B), which is involved with the expression of genes of oxidant and inflammatory compounds, shows a great activation in the immune cells in situations of oxidative stress (83), as happens in aging. This inflammation/oxidation theory of aging is supported by the fact that the immune system, with the passage of time, has had to face numerous foreign agents, thereby producing more and more oxidants and inflammatory compounds with resulting chronic oxidative stress. As a result of the oxidative injury that immune cells suffer with age, these cells may lose some of their capacity to regulate their own redox balance, which would result in a "vicious circle" in which factors such as NF- κ B could be implicated, stimulating the oxidative stress even more, as shown in Fig. 1.

HYPERTENSION AND AGING

There is considerable evidence that hypertension is closely linked to aging, and both normal senescence and high blood pressure adversely affect the cardiovascular system (80). As hypertension generally seems to increase cardiovascular aging in humans and experimental animals, it is often considered an accelerated form of vascular aging (41). Accordingly, several studies have shown that wall thickness increases with age and that age-related hypertension is linked to arterial stiffness (45).

The importance of cardiovascular diseases in the elderly is based on the fact that they are the most frequent cause of death among persons over 65 years, as well as being responsible for the major proportion of hospitalizations and health care costs for the aged (54, 61). Moreover, the importance of cardiovascular diseases increases along with the growth of

TABLE 1. CHANGES WITH AGING OF OXIDATIVE STRESS PARAMETERS IN PERITONEAL LEUKOCYTES FROM MICE: EFFECTS OF A DIET SUPPLEMENTED WITH ANTIOXIDANTS

	<i>Aging</i>	<i>Antioxidant supplementation</i>
<i>Oxidant and proinflammatory compounds</i>		
Extracellular superoxide anion	Increase	Decrease (= adult)
GSSG	Increase	Decrease (= adult)
GSSG/GSH	Increase	Decrease (= adult)
TNF α	Increase	Decrease (= adult)
PGE ₂	Increase	Decrease (= adult)
<i>Antioxidant defenses</i>		
GSH	Decrease	Increase (= adult)
SOD	Decrease	Increase (= adult)
CAT	Decrease	Increase (= adult)
GPx	Decrease	Increase (= adult)
GR	Decrease	Increase (= adult)
<i>Oxidative damage</i>		
MDA	Increase	Decrease (= adult)
8oxodG	Increase	Decrease (= adult)

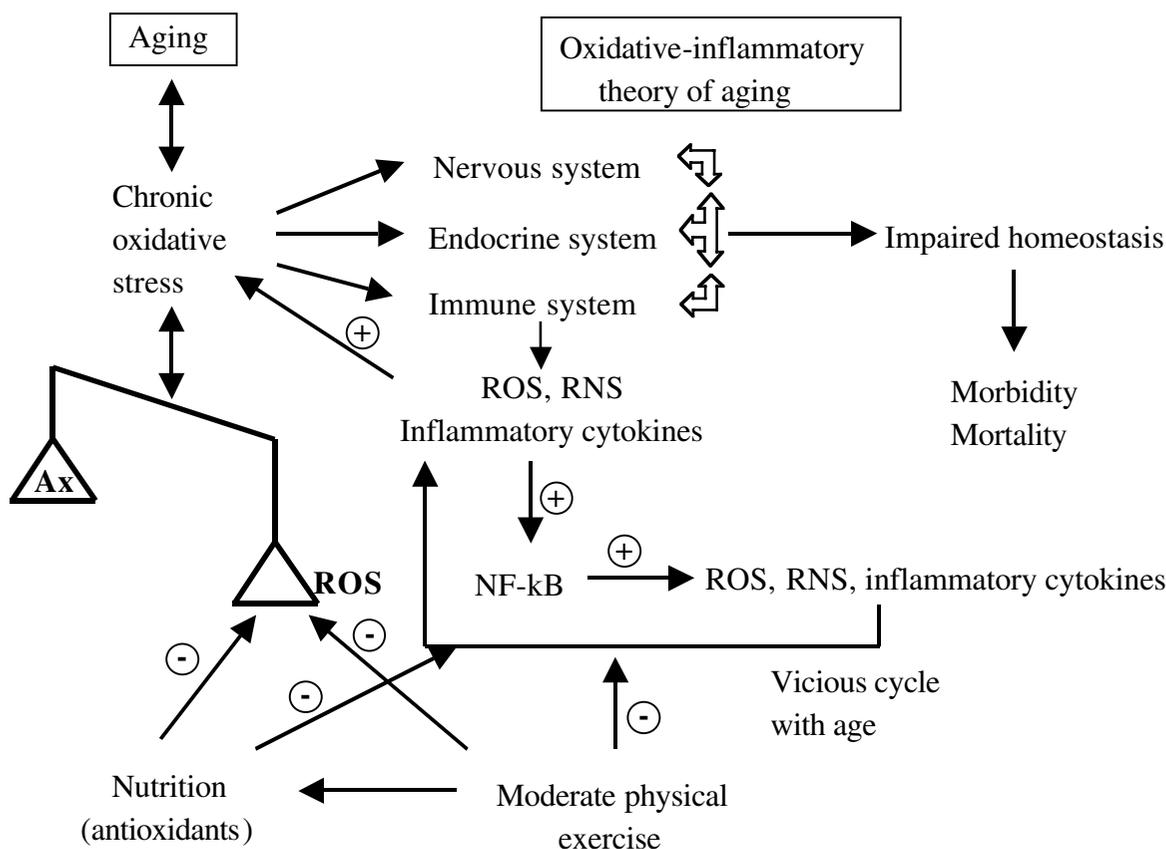


FIG. 1. An approach to possible mechanisms involved in the oxidative/inflammatory theory of aging: role of nutrition (antioxidants) and moderate physical exercise. We suggest that aging is a chronic oxidative stress with higher levels of oxidant compounds such as ROS and lower antioxidant (Ax) defenses. This imbalance in the oxidant/antioxidant levels is responsible for the changes with age in the nervous, endocrine, and immune systems and in their communications, these changes being the causes of the impaired homeostasis and increasing morbidity and mortality that occur with aging. The immune system participates in this oxidative stress because it produces in its function elevated levels of oxidants [ROS and reactive nitrogen species (RNS)] and inflammatory cytokines, which stimulate the activation of NF-κB. This nuclear factor produces more oxidants and inflammatory compounds, establishing a vicious circle that increases with age. Nutrition (specially the antioxidant compounds of the diet) and moderate physical exercise (by itself and through its positive effect on antioxidant defenses) are two good strategies for neutralizing the chronic oxidative stress of aging.

the elderly population. As a matter of fact, largely due to a better control of infectious diseases and access to a more nutritious diet, life expectancy in developed countries has increased dramatically in the 20th century, with the resulting increase in the number of the elderly population. The increase in the mean age of the population is accompanied by a considerable incidence of chronic age-related processes, such as the so-called free-radical diseases, which include cancer, atherosclerosis, essential hypertension, and cardiovascular and Alzheimer diseases (31, 42). Numerous epidemiological studies have clearly demonstrated that the incidence of hypertension increases over 30–65% in the elderly, with these elderly hypertensive patients being much more vulnerable to stroke, myocardial infarction, congestive heart failure, and renal failure than their younger counterparts (5).

It is evident that oxidative stress is involved in both hypertension and aging. In fact, the use of biological markers of aging has been proposed to provide a better understanding of the etiology of hypertension (2). There is evidence in humans that both hypertension and aging impair endothelial function

and that a superoxide anion excess could be a common cause of endothelial dysfunction (28). Moreover, oxidative stress seems to be implicated in the age-related decrease of nitric oxide-mediated vascular relaxation in hypertensive rats (SHR) (58), and arteries from old SHR show an increased superoxide anion production compared with those from young animals (17). As we have previously confirmed that several functional immune parameters change with age (7), we have studied these parameters in immune cells from hypertensive women in comparison with normotensive controls of the same age (65 ± 5 years). As shown in Figs. 2–5, functions that decrease with age, such as chemotaxis of neutrophils and lymphocytes (Fig. 2), lymphoproliferative response to phytohemagglutinin (PHA) mitogen (Fig. 3), IL-2 release by these lymphocytes (Fig. 4), and NK activity against tumoral cells (Fig. 5), are more reduced in hypertensive than in normotensive women. Thus, in agreement with the above comments, and as could be inferred from our experimental results (Figs. 2–5), it is reasonable to maintain that hypertensive women are biologically older than their normotensive counterparts.

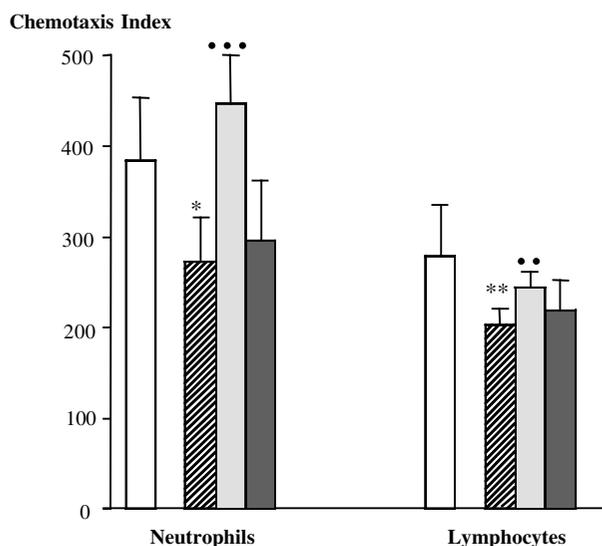


FIG. 2. Chemotaxis index (number of cells that pass the filter in a Boyden chamber with F-met-leu-phe as chemoattractant) of peripheral blood neutrophils and lymphocytes from sedentary normotensive elderly women (65 ± 5 years old) (blank columns) and hypertensive women of the same age (hatched columns). The effects on this function in hypertensive women of a moderate exercise program for 6 months are shown in the gray columns. The black columns show the data corresponding to the values obtained at 6 months after finishing the exercise program, without any subsequent exercise. Each value is the mean \pm SD of 10 experiments performed in duplicate. * $p < 0.05$; ** $p < 0.01$ with respect to the corresponding values in normotensive women. * $p < 0.01$, *** $p < 0.001$ with respect to the corresponding values before starting the exercise program.

ANTIOXIDANTS, OXIDATIVE STRESS, AND IMMUNE CELL FUNCTIONS IN AGING AND HYPERTENSION

As we have already pointed out, there is a great amount of data suggesting the existence of an oxidant-antioxidant imbalance in aging, as well as in hypertension. The first observations suggesting a key role for oxidative injury in hypertension and aging were those showing the favorable effects of endogenous and dietary antioxidants (20), which have been demonstrated in experimental animals and human subjects (36, 49). Moreover, it has been shown that antioxidant or anti-inflammatory therapy and inhibition of lipid peroxidation processes prevent some vascular alterations associated with aging and restore normal blood pressure values in hypertension. A direct link between oxidative stress, aging, and human diseases can be further established by studies analyzing the impact of variation in human genes encoding for antioxidant defense systems (18). The endogenous antioxidants decrease in oxidative stress situations, such as endotoxemia, hypertension, and aging, because they are spent neutralizing the excess of ROS. Thus, the oxidant-antioxidant imbalance in hypertension and aging can be reflected by a deficiency of GSH (34). In fact, studies performed in aged animals and elderly

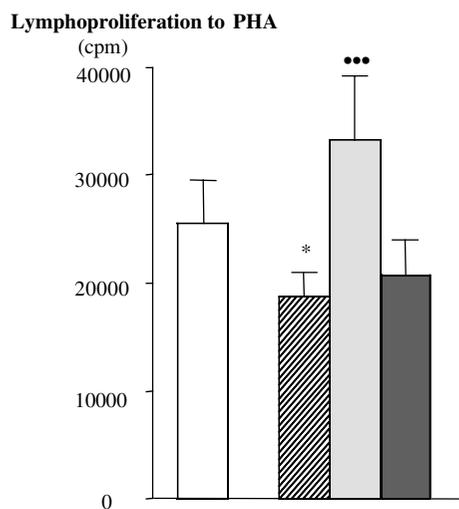


FIG. 3. Lymphoproliferation in response to the mitogen PHA (cpm obtained in cultures of 3 days with [3 H]-thymidine added 24 h before the end of culture) by peripheral blood mononuclear cells from sedentary normotensive elderly women (65 ± 5 years old) (blank column) and hypertensive women of the same age (hatched column). The effects on this function in hypertensive women of a moderate exercise program for 6 months are shown in the gray column. The black column shows the data corresponding to the values obtained at 6 months after finishing the exercise program, without any subsequent exercise. Each value is the mean \pm SD of 10 experiments performed in duplicate. * $p < 0.05$ with respect to the corresponding values in normotensive women. *** $p < 0.001$ with respect to the corresponding values before starting the exercise program.

people showed a decrease in the GSH levels, in plasma, blood, organs, and immune cells of human and experimental animals (11, 32). In addition, the plasma MDA levels showed an increase in aging rats, which was inversely related to plasma GPx activity (68) and erythrocytic GSH content, as well as to SOD, CAT, and GPx activities in red blood cells (87). At the vascular level, a decrease in the antioxidant capacity is associated with the accumulation of MDA in cerebral microvessels from aged rats (51). However, there are also conflicting results. Thus, high plasma MDA levels in healthy humans correlated with high activity of CAT and GPx in erythrocytes, but with lower SOD activity in the most aged group studied (33). Nevertheless, it is generally accepted that longevity may be associated with an optimal antioxidant protection, and that the senescent decrease in antioxidant levels supports the free radical theory of aging and provides a rationale for attempts to decrease the rate of aging by supplementing the diet with antioxidants (7, 49).

Previous studies from our laboratory on old mice and elderly men and women have demonstrated the beneficial effect *in vitro* and *in vivo* on the immune functions of antioxidants such as vitamin E, vitamin C, GSH, *N*-acetylcysteine (NAC), or thioproline (TP) (7). Moreover, we have observed that supplementation of the diet with antioxidants, such as NAC and TP and other mixes of antioxidants (vitamin C, vitamin E, β -carotene, zinc, and selenium), not only improves the immune function in aging mice, bringing it to adult values,

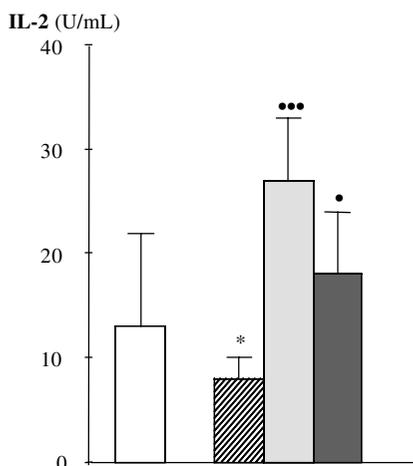


FIG. 4. IL-2 release in the above-mentioned cultures (U/ml obtained using an ELISA method) by peripheral blood mononuclear cells from sedentary normotensive elderly women (65 ± 5 years old) (blank column) and hypertensive women of the same age (hatched column). The effects on this function in hypertensive women of a moderate exercise program for 6 months are shown in the gray column. The black column shows the data corresponding to the values obtained at 6 months after finishing the exercise program, without any subsequent exercise. Each value is the mean ± SD of 10 experiments performed in duplicate. **p* < 0.05 with respect to the corresponding values in normotensive women. †*p* < 0.05, ****p* < 0.001 with respect to the corresponding values before starting the exercise program.

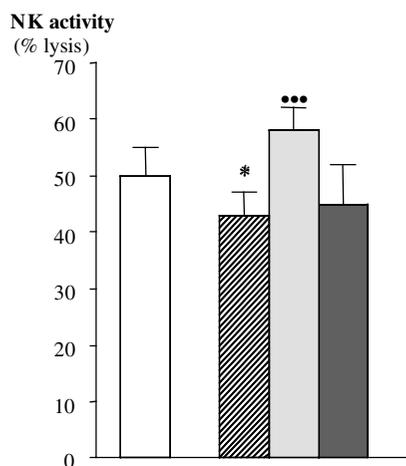


FIG. 5. NK activity (% lysis of human tumoral K562 cells) by peripheral blood mononuclear cells from sedentary normotensive elderly women (65 ± 5 years old) (blank column) and hypertensive women of the same age (hatched column). The effects on this function in hypertensive women of a moderate exercise program for 6 months are shown in the gray column. The black column shows the data corresponding to the values obtained at 6 months after finishing the exercise program, without any subsequent exercise. Each value is the mean ± SD of 10 experiments performed in duplicate. **p* < 0.05 with respect to the corresponding values in normotensive women. ****p* < 0.001 with respect to the corresponding values before starting the exercise program.

but also neutralizes the oxidative stress, thus helping to reach values of oxidants, antioxidants, and biomolecular damage similar to those of adults (results summarized in Table 1). In unpublished work from our laboratory, we have also found that mice with an antioxidant-supplemented diet show a significant increase in their life span.

As can be seen in the results recently obtained by our group and shown in Table 2, the ingestion by elderly women (65 ± 5 years old) of vitamin C (500 mg) and vitamin E (200 mg) daily for 3 months improves all the functions studied in peripheral blood immune cells. Thus, chemotaxis of neutrophils

and lymphocytes, phagocytosis of neutrophils, lymphoproliferative response to the mitogen PHA, and NK cytotoxicity, functions that decrease with aging, are higher after supplementation, which brings them close to the values of these activities in adult women (30 ± 5 years). In a similar way, adherence of neutrophils and lymphocytes, a function that increases with aging, is diminished after antioxidant intake, reaching the values of adult women. It is possible that the beneficial effect of this supplementation on the immune system is carried out through a decrease of the oxidative stress in these cells, in a similar way to that observed in leukocytes

TABLE 2. EFFECTS OF DAILY VITAMIN C (500 MG) AND VITAMIN E (200 MG) SUPPLEMENTATION FOR 3 MONTHS ON SEVERAL IMMUNE FUNCTIONS IN ELDERLY WOMEN

	Elderly women		Adult women
	Before	After	
Neutrophil adherence index	61 ± 8	47 ± 7†	44 ± 6
Neutrophil chemotaxis index	378 ± 84	569 ± 103‡	584 ± 95
Neutrophil phagocytosis index	140 ± 36	229 ± 50‡	203 ± 36
Lymphocyte adherence index	52 ± 9	36 ± 9†	42 ± 9
Lymphocyte chemotaxis index	241 ± 48	319 ± 68†	331 ± 62
Lymphoproliferation to PHA (cpm)	13,802 ± 3,630	40,385 ± 14,368‡	37,849 ± 11,653
NK activity (% lysis)	51 ± 12	63 ± 10*	58 ± 12
IL-2 release (U/ml)	13 ± 4	25 ± 8†	21 ± 6

Each value is the mean ± SD of 10 experiments performed in duplicate.

**p* < 0.05, †*p* < 0.01, and ‡*p* < 0.001 with respect to the corresponding values before supplementation.

from mice. As the changes in the immune functions found with antioxidant supplementation are similar in humans and mice, and because these changes in mice are accompanied by an increase in the life span, it is probable that similar effects could be obtained in humans.

As occurs with respect to aging, controversial results related to the differences in the antioxidant status, as well as the levels of oxidants and MDA, between SHR and patients and their respective normotensive controls have been described. In the SHR, the antioxidant defenses of erythrocytes, hepatocytes, and myocardium may be lower or higher than, or similar to, those of control animals (37), whereas the hypertensive patients have lower plasma, serum, and erythrocyte GSH levels, as well as lower erythrocyte GPx activity, as compared with normotensive subjects (60). Moreover, in plasma of hypertensive patients, an enhancement of MDA levels associated with a decrease in SOD activity (70) has been observed, and in leukocytes from these patients a high production of superoxide anion and hydrogen peroxide has been found (39). However, in plasma and in erythrocytes from hypertensive subjects, as compared with those from the normotensives, a similar total antioxidant capacity (38) and activities of GPx and CAT (39) or increase in GPx activity (70) have also been described. Nevertheless, antioxidants, such as vitamin E, GSH, NAC, melatonin, or SOD, may normalize blood pressure, restore vascular function, and decrease oxidant levels and oxidative damage to biomolecules in hypertension (53, 69, 81). An antioxidant-enriched diet reduces the renal inflammation and relieves hypertension in SHR (66). Moreover, melatonin has been shown to ameliorate hypertension in SHR in association with a reduction in the activation of NF- κ B, probably as the result of a reduction in oxidative stress, inflammation, and renal immune infiltration (53). In addition, there is evidence that some antihypertensive drugs, such as angiotensin inhibitors, calcium entry blockers, or β -blockers, probably act not only by reducing blood pressure, but also because of their antioxidant properties (6, 72).

PHYSICAL EXERCISE IN AGING AND HYPERTENSION: EFFECTS ON THE IMMUNE SYSTEM

Physical activity is deemed to have a beneficial effect on health. For this reason, it is one of the lifestyle factors that can improve health and quality of life in elderly persons (62). There is a wealth of information on the effects of physical exercise on the immune function of adult experimental animals and humans. Although conflicting results have been obtained, depending on the type of exercise, immune function studied, or state of the subject, it is generally accepted that an acute or very strong training induces an inflammatory response with selective activation or depression of immune cell functions, whereas moderate training exercise leads to adaptations of the immune cells with improvement of their functions (59). However, in old animals or elderly humans, the effects of physical exercise on the immune functions have been very scarcely studied. Considering what has already been said on the deterioration of the immune system in old age, and the

available data on the effects of exercise on the immune system, it seems that the practice of moderate exercise is an important candidate for improving the immune function in the elderly. In fact, several authors and our own group have shown that, in old animals, regular exercise training improves immune functions (16, 59). In addition, we have observed that the favorable effects of exercise are higher in old than in young mice (16). In previous work, several authors have found that the practice of regular physical exercise maintains a better immune system in elderly subjects, but exercise should be performed for a period longer than 4 months in order to observe a significant improvement of the immune functions (64). We have observed (unpublished observations) that elderly women (60 ± 5 years old) who performed a moderate physical exercise for 6 months (three sessions of 45 min per week) improved significantly their immune system as regards neutrophil, lymphocyte, and NK functions. A limited number of similar studies from other laboratories obtained comparable results (59, 73).

The beneficial effects on health of regular physical exercise include a decrease in the blood pressure level, especially in hypertensive patients, and therefore it is accepted that exercise is effective for the prevention and treatment of hypertension (84). Three exercise sessions per week have been considered to be the minimal frequency for significant blood pressure reduction. High-intensity exercise [$>75\%$ VO₂ (max)] may not be as effective as low-intensity exercise [$<70\%$ VO₂ (max)] for reducing elevated blood pressures. However, not all hypertensive patients respond to the exercise treatment (84). Moreover, some subjects show a more pronounced blood pressure response to endurance training than others, despite identical training programs and similar initial blood pressure levels. This is an example of normal biological diversity. Data from epidemiologic studies indicate the role of genetic factors in the modification of blood pressure responses to endurance training (65). It is a fact that lowering the blood pressure in older adults reduces their cardiovascular risk, and this can be accomplished with low-intensity endurance exercise training, although the metabolic adaptations to this exercise can significantly decrease other risk factors for coronary artery disease and atherosclerosis, in addition to reducing blood pressure (14). Thus, the favorable effects of exercise training go beyond the recognized benefit of blood pressure reduction (75). Exercise training, but not that performed by elite athletes (40), may improve health and prevent cardiovascular diseases, with the strongest benefit being the effect on endothelial vasodilator function (75). Physical activity does not need to be vigorous to benefit health. It is moderate activity, such as brisk walking for 30–60 min a day most days of the week, which is associated with a significant reduction in the incidence and mortality of cardiovascular diseases (26).

In the last years, the hypothesis has been increasingly accepted that physical exercise modulates immune cell functions, which are involved in atherosclerosis and therefore in the related diseases, and that, through this modulation, exercise is capable of protecting against atherosclerosis or inducing regression of atherosclerotic lesions (19). However, no data are available on the effect of exercise on the immune functions in hypertensive subjects. For this reason, we have

examined in a group of 10 hypertensive women the effect of a 6-month exercise program (mentioned above) on several immune cell functions. After this time, the moderate intensity exercise improved immune functions such as the chemotaxis of neutrophils and lymphocytes (Fig. 2), as well as the lymphoproliferative response to the mitogen PHA (Fig. 3), IL-2 release (Fig. 4) and NK activity (Fig. 5). These functions were reduced in hypertensive elderly women, even more than in normotensive women of the same age, who showed these functions very decreased with respect to those of adult women. A moderate physical exercise performed for 6 months increased these functions, with the values becoming similar to those of normotensive women. After 6 months without performing exercise, the above-mentioned functions went back to the initial preexercise values. In previous studies, it has been observed that a 6-month exercise program decreased the production of atherogenic cytokines such as IL-1 α , TNF α , and interferon- γ , and increased the release of atheroprotective cytokines such as IL-4, IL-10, and transforming growth factor β 1 by blood mononuclear cells (74).

It is accepted that strenuous physical exercise may be a significant oxidative stress because increased consumption of molecular oxygen for respiration may generate higher amounts of ROS. According to several reviews of the broader field of exercise and oxidative damage (63), vigorous exercise is accompanied by the involvement of immune cells, especially phagocytic cells, in the generation of oxidants through the activation of factors such as NF- κ B (55). However, as reviewed by Johnson (35), in response to repetitive or graded exercise training, a decrease in oxidative stress and a resistance to oxidative damage appear. This fact may be due to exercise-induced changes in antioxidant enzymes not only in the skeletal muscle, which is recognized as a major source of free radical generation, but also in other tissues and cells. Although the results show discrepancies, depending on the species, tissue, type and subtype of cell, age of animal, and training regimen, in general, an up-regulation of antioxidant defenses appears with moderate exercise training (35). As regards the immune cells, the down-regulation of the release of ROS and the adaptation of antioxidant mechanisms to regular exercise have been observed in phagocytic cells by us and other authors (8, 55). In fact, we found that the ascorbate content in macrophages increases after exercise in both young and old mice (8). Moreover, the results obtained by us (Table 3) show that a moderate physical exercise as that performed

by students of the National Institute of Physical Education [Instituto Nacional de Educación Física (INEF), in Spain] improves functions of peripheral blood neutrophils and lymphocytes, such as phagocytic capacity and lymphoproliferative response to mitogen PHA, respectively, and increases antioxidant defenses as shown by the higher levels of vitamins C and E and the increased SOD activity with respect to the values obtained in sedentary students. It is possible that moderate exercise in hypertensive animals and humans causes similar changes in the antioxidant defenses of their immune cells, and that this fact could be, in part, responsible for their improved function.

CONCLUSIONS

In summary, both aging and hypertension are related to chronic oxidative stress, *i.e.*, to an imbalance in the antioxidant/oxidant levels with an increase in ROS production and a decrease in antioxidant defenses. This chronic oxidative stress injures cells and systems, but the effects are more evident in the regulatory systems, such as the nervous, endocrine, and immune systems. These systems communicate in a bidirectional way (1), and presently it is accepted that there is a “neuroimmunoendocrine system” that allows the maintenance of homeostasis in the organism and therefore preserves health. Oxidative stress could alter this communication, and in fact, there is a theory of aging proposing the disturbed communications as the cause of health loss with age (15). Our own data show errors in the communication between the nervous and the immune system with aging (9). This alteration could explain the impaired homeostasis that leads to an increase in the morbidity and mortality of the aged. Focusing on the immune system, which as mentioned above produces oxidants and inflammatory compounds in its daily work, the altered communication results in a vicious circle that maintains the state of chronic oxidative stress. An adequate nutrition, with antioxidant supplementation, and moderate physical exercise can neutralize, in part, the oxidative stress and the vicious circle of oxidation produced by the immune cells. As regards physical exercise, this favorable effect could be mediated by factors produced in the physical activity, as well as through an increase in the antioxidant defenses in cells, in general, and in the immune cells, in particular. Thus, as shown in Fig. 1, antioxidant ingestion and moderate

TABLE 3. LEVELS OF VITAMIN C AND VITAMIN E AND SOD ACTIVITY IN PERIPHERAL LYMPHOCYTES AND NEUTROPHILS, AS WELL AS SPECIFIC FUNCTIONS OF THESE CELLS, FROM SEDENTARY SUBJECTS AND SUBJECTS PERFORMING HABITUAL MODERATE EXERCISE

	<i>Lymphocytes</i>		<i>Neutrophils</i>	
	<i>Sedentary</i>	<i>Exercise</i>	<i>Sedentary</i>	<i>Exercise</i>
Vitamin C (nmol/10 ⁸ cells)	135 ± 21	326 ± 62 [†]	164 ± 24	194 ± 31*
Vitamin E (nmol/10 ⁸ cells)	620 ± 172	2,062 ± 297 [‡]	1,520 ± 250	2,167 ± 660*
SOD (U/10 ⁸ cells)	20 ± 6	42 ± 10 [‡]	10 ± 2	32 ± 5 [‡]
Lymphoproliferation to PHA (%)	3,425 ± 596	4,203 ± 740*		
Phagocytosis index			120 ± 18	230 ± 42 [‡]

Each value is the mean ± SD of 10 experiments performed in duplicate.

**p* < 0.05, [†]*p* < 0.01, and [‡]*p* < 0.001 with respect to the corresponding values in sedentary subjects.

physical exercise could be two useful strategies to control oxidative stress and, consequently, to revitalize the immune system, with resulting improvement in health and increased longevity.

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ABBREVIATIONS

CAT, catalase; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; IL, interleukin; LPS, lipopolysaccharide; MDA, malondialdehyde; mtDNA, mitochondrial DNA; NAC, *N*-acetylcysteine; NF- κ B, nuclear factor- κ B; NK, natural killer; 8oxodG, 8-oxo-7,8-dihydro-2-deoxyguanosine; PAM, prematurely aging mice; PGE₂, prostaglandin E₂; PHA, phytohemagglutinin; ROS, reactive oxygen species; SHR, spontaneously hypertensive rats; SOD, superoxide dismutase; TNF α , tumor necrosis factor- α ; TP, thioprolone.

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