Dietary vitamin E and physical exercise: II. Antioxidant status and lipofuscin-like substances in aging rat heart

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Abstract

The heart faces a high risk of free radical injury owing to a slow generation of antioxidant (AO) enzymes by its cells. A general decline in this system may be another reason for the development of age-related diseases. Although the correlation between aging and exercise has been studied extensively, these studies have produced conflicting data on the effects of vitamin E on the aging heart, when it is introduced as an intervening factor. To investigate these effects, we determined the activities of antioxidant enzymes (AOEs) such as superoxide dismutase (SOD) and catalase (CAT), lipid peroxidation (LP), lipofuscin (LF)-like autofluorescent substances and vitamin E content in the left and right ventricles (LV and RV) of the heart in male Wistar albino rats of 4-(young adults), 8-(old adults), 12-(middle-age) and 22-mos(old) of age. Animals were orally supplemented with vitamin E and allowed to swim for 30 min/day, 5 days/week and for a total period of 60 days. Exercise training in all the age groups except the old was effective in upregulating the SOD activity. Old trainees showed an increase in SOD activity when supplemented with vitamin E. In the 22-mo-olds, a remarkable decrease in CAT activity was seen. Exercise by itself upregulated the CAT as well as SOD activity in all age groups except the old wherein vitamin E was effective in increasing the activities of AOEs. Supplementation significantly reduced LP as evidenced by lowered malondialdehyde (MDA) and LF-like autofluorescent substances in the trained as well as sedentary rats. Tissue vitamin E content was low in the swim trainees that were not supplemented. This change, well emphasized in the trainee groups of 22-mo-old suggests the probable utilization of vitamin E in keeping free radicals at bay. Our results suggest that vitamin E can stand out as a significant tool in ameliorating the declining AO defense in the old rats.

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1. Introduction

During exercise there is an increased utilization of oxygen as a metabolic fuel, which would mean an increase in turnover of reactive oxygen species (ROS) (Asha Devi, 2002; Sen, 1995), extensive lipid peroxidation (Hemnani and Parihar, 1998) and extensive damage to biological macromolecules (Halliwell et al., 1992). Paradoxically, the heart, an organ which has post-mitotic cells and one of the highest oxygen consumption rates in the body, has a slow turnover of antioxidant enzymes (AOEs) and is therefore, highly susceptible to ROS (Somani, 1996). The impact of ageing on the myocardial antioxidant (AO) function has been drawing increased attention, more so after

the discovery of the role of ROS in the etiology of ischaemic reperfusion (IR) injury of the heart. Vitamin E has been considered in our studies because of its close association with cell membranes and it is used in conjunction with swim training because (a) their interaction can suggest an increase in the endogenous defenses when compared with their sedentary counterparts and (b) vitamin E has inhibitory effects on the storage of lipofuscin-like autofluorescent substances in the primary cell cultures of rat myocytes (Asha Devi et al., 1990) as well as neurons (Kan et al., 1991).

In this study, swimming was chosen as a suitable method of exercise in rats, since this type of exercise exposes the heart to work overload and activates synthesis of proteins (Bonnin et al., 1981). Rodents are good swimmers with negligible emotional disturbances under natural conditions. Besides, electric-stimulation and plyometric contractions as

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seen on a treadmill can be minimized since these factors themselves act as inducers of oxidative stress (Radak et al., 1999).

Earlier studies have indicated age-related biochemical adaptations in the myocardium of adult and middle-aged rats that were moderately swim trained (Asha Devi et al., 2002a; Anitha and Asha Devi, 1996) although to a lesser extent in the old. Also the effects of aging in rats are known to appear first on the left side of the heart leading to left side dysfunction and failure (Anversa et al., 1986).

Our studies on the plasma lipid profile in rats have shown a positive response in that swim exercise with vitamin E intake can lower LDL-C and cholesterol while it elevates the HDL-C level even in the old rats (Asha Devi et al., 2002b). Although available reports do exist to support the fact that trained exercise and AOs are beneficial to the heart, regional and age-related variations in the rats' AO system due to swim exercise, when initiated at different ages, and in combination with vitamin E have not been investigated. The present study was based on three hypotheses: (1) although swim training of moderate intensity and duration, when initiated at various ages, may ameliorate the rats' defense mechanism, it by itself may not prove beneficial with advancing age. For this purpose, we analyzed the activities of superoxide dismutase (SOD) and catalase (CAT) in order to document the rats' endogenous AO system. (2) Exercise, when coupled with a dietary AO, may be more effective in checking the free radical damage. For this we have examined the efficacy of vitamin E in checking lipid peroxidation. (3) The extent of protection offered by exercise and AO supplementation in normal myocardial aging may also be region-specific. The left and right ventricles were analyzed separately to characterize the regional responses.

2. Materials and methods

The present study was approved by the Institutional Animal Ethics Committee (IAEC), Bangalore University, India.

2.1. Animal care and training protocol

Male pathogen-free albino rats of Wistar strain of 4 months (mos) old were obtained from the Central Animal Facility, IISc, Bangalore and were maintained until they were 8-, 12- and 22-mos in a clean rodent room without the SPF status being monitored. However, no animals from outside were allowed and the room was exclusively meant for maintenance of aging rats. The maximum length of life of male Wistar rats in our animal colonies has been 30 months with the median length of 15.5 months. Experiments were completed taking in to consideration the entire age-scale encompassing the young adult, old adult, middle-aged and the old. Animals were housed 2–3 per cage in cages that

were fitted with stainless-steel wire-mesh bottoms, at the temperature of $28 \pm 1^\circ\text{C}$, relative humidity of $17 \pm 1\%$ and under a daily photoperiod of 12 h-light and 12 h-dark cycle. The animals were fed with lab chow (Lipton India Ltd Bangalore) and tap water ad libitum. Rats were assigned to (A) two sedentary groups—controls without (SE-C, N) and controls with vitamin E supplementation (SE-C, +E), and, (B) two swim trained groups—swim trainees on a normal diet (SW-T, N) and swim trainees on vitamin E supplementation (SW-T, +E). At the beginning of the training regimen, the mean body weights of various groups did not differ significantly. Exercise training was similar to protocol described in Asha Devi et al. (2002a) with minor modifications. Briefly, animals were exercised in a glass tank ($77 \times 38 \times 39$ cm) filled to a depth of 21 cms for rats of 4 and 8 mos, 25 cms for those of 12 mos and 31 cms for those of 22 mos. Rats were made to swim with 3% of their body weight tied to their tails. Initially they were made to exercise for 5 min/day with a progressive increase to 30 min/day over a period of 1 week, and thereafter for 30 min/day for a total training period of 12 weeks with five training days/week. Two groups of animals received a daily oral supplementation of 50 IU/kg of α -tocopherol, throughout the training period. The group SE-C(N) under (A) was restricted to cage activity. No deaths occurred during or after exercise in any of the groups in any age.

Tissue preparation. All animals were sacrificed under light ether anesthesia. Preparation of the left and right ventricles was similar to our earlier protocol (Prathima and Asha Devi, 1999). One part of the divided regions taken from the same region in the heart of each animal was minced, washed in the medium and homogenized in ice-cold 50 mM phosphate buffer containing 0.1 mM EDTA (pH 7.4). Tissue homogenates were centrifuged at 1000 rpm for 10 min at 4°C (RV/FM Superspin, Plastocrafts, India). The supernatant was used for analytical procedures.

2.1.1. Analytical procedures

Chemicals and solvents. α -Tocopherol, *t*-butyl hydrogen peroxide, epinephrine, thiobarbituric acid, pyridine, triton X-100, and BSA were obtained from Sigma Chemicals (St Louis, MO). All organic solvents were of spectral grade and general chemicals were of reagent grade.

Enzyme assays. *Catalase* (CAT, EC 1.11.1.6). CAT was determined by the method of Aebi (1984). Briefly, 100 μl of the tissue supernatant with an equal volume of absolute alcohol was incubated for 30 min at 0°C following which triton X-100 was added. A known volume of this was taken in an equal volume of 0.066 M H_2O_2 in phosphate buffer and the decrease in absorbance measured at 240 nm for 30 s in a spectrophotometer (ELICO, Model SL 159). An extinction coefficient of 43.6 M cm^{-1} was used to determine enzyme activity, one unit of which is equal to the moles of H_2O_2 degraded/min/mg of protein.

Superoxide dismutase (SOD, EC 1.15.1.1). SOD activity was determined by the method of Misra and

Fridovich (1972). The amount of enzyme that results in 50% inhibition of epinephrine autooxidation is defined as one unit.

Lipid peroxidation (LP). This assay was used to analyze malondialdehyde (MDA) level by the method of Ohkawa et al. (1979) using 1,1,3,3-tetramethoxypropane (TMP) as the standard. LP was expressed as *n* moles MDA/mg of protein.

Tissue vitamin E. The quantity of vitamin E in the tissues was determined by the method described by Desai (1984). Briefly, LV and RV were isolated and homogenized in five volumes of isotonic KCl. The saponification mixture consisted of tissue extract and purified absolute alcohol, to which was added 25% ascorbic acid and then pre-incubated at 70 °C. The mixture was treated with 10N KOH and cold hexane to extract the non-saponifiable material. Hexane layer was separated at 1500 rpm for 10 min and a known volume of this extract was evaporated to dryness. The residue was then dissolved in chloroform and spotted on a set of duplicate silica gel G plates. Pure tocopherol standard was also spotted and the plates were developed using 2:1 benzene–ethyl acetate. The reference plate was sprayed with 0.001% rhodamine 6G in purified methanol to identify the spots under UV light and from the other plate the samples were eluted in absolute ethanol, centrifuged at 2500 rpm for 5 min. Vitamin E in the eluate was quantified using bathophenanthroline, ferric chloride and orthophosphoric acid. Samples were read at 536 nm and the quantity of vitamin E was expressed in terms of µg/g tissue.

Measurement of lipofuscin (LF)-like autofluorescent material. Fluorescent substances in the LV and RV were measured by the method of Shedahl and Tappel (1974). Briefly, tissue homogenates were prepared in 2:1 (V/V) chloroform: methanol and centrifuged at 3000 rpm for 10 min at 4 °C. The pellet was suspended in the same solvent mixture and centrifuged at 3000 rpm for 10 min at 4 °C. The supernatants were combined and recentrifuged at 6000 rpm for 10 min. The lower chloroform layer was aspirated and fluorescence was measured at an excitation wavelength of 366 nm and emission wavelength of 485 nm using quinine sulfate in 0.1N sulfuric acid in a spectrofluorimeter (Genway, Model 6200, UK). Autofluorescence intensity was expressed as units/100 mg tissue.

Statistical analyses. All the data were expressed as means ± SEs and were analyzed within a two-factor analysis of variance (ANOVA) between groups and regions. A three-way ANOVA was performed for effects of age, exercise and region over AO enzymes, MDA, LF-containing fluorescent material and vitamin E levels. When a significant *F* ratio was found, Duncan's multiple range tests (DMRT) were used to assess the differences between group means. Probability values (*P*) < 0.05 were considered significant (Snedecor and Cochran, 1994).

3. Results

3.1. Tissue antioxidants

CAT activity in 4-mo-old animals was highlighted by a significant two-fold increase in the LV of SW-T(+E) compared to SE-C(N). Increase in activity was also seen in the LV and RV of other experimental groups, but to a lesser extent (SE-C(+E), LV-87% and RV-55%; SW-T(N), LV-61% and RV 31%). In the 8-mo-olds, the trained groups, more so SW-T(+E) showed a higher CAT activity than SE-C(N). Regional differences were insignificant in the 12-mo-old animals. SW-T(+E) and SW-T(N) exhibited higher CAT activity in this age group too. In the 22-mo-olds, the two trained groups did not differ significantly in their CAT activities (Table 1).

In the 4-mo-olds, increase in SOD activity was similar in extent in both the trained groups. In 8-mo-olds, the swim trainees supplemented with vitamin E showed 31 and 17% greater SOD activity in the LV and RV, respectively. However, SW-T(N) did not reveal significant changes compared to their sedentary counterparts. An overall reduction in SOD activity was seen in the 12-mo-olds compared to the two preceding age groups. SW-T(N) exhibited a lesser extent of increase than the trainees supplemented with vitamin E. In the 22-mo-olds, SW-T(N) recorded a 15% lower activity in both the regions while

Table 1

Catalase activity as a function of age, exercise and dietary vitamin E supplementation

Age(mos)	Groups	LV	RV
4	SE-C(N)	63.82 ± 9.61 ^A	71.30 ± 7.99 ^A
	SW-T(N)	102.55 ± 16.18 ^{BC}	93.13 ± 16.19 ^{BC}
	SE-C(+E)	119.48 ± 2.00 ^{CD}	110.69 ± 2.54 ^{CD}
	SW-T(+E)	146.85 ± 2.22 ^{DE}	104.23 ± 3.06 ^{DE}
8	SE-C(N)	84.64 ± 4.47 ^A	89.11 ± 3.36 ^A
	SW-T(N)	112.71 ± 1.09 ^B	103.05 ± 2.00 ^B
	SE-C(+E)	118.35 ± 2.49 ^B	83.12 ± 1.30 ^B
	SW-T(+E)	142.54 ± 8.66 ^C	104.17 ± 0.49 ^C
12	SE-C(N)	90.47 ± 0.64 ^A	75.69 ± 2.47 ^A
	SW-T(N)	105.48 ± 1.30 ^B	112.11 ± 1.57 ^B
	SE-C(+E)	105.62 ± 0.51 ^B	111.88 ± 2.17 ^B
	SW-T(+E)	124.93 ± 2.33 ^C	125.18 ± 1.36 ^C
22	SE-C(N)	74.97 ± 1.73 ^A	78.67 ± 2.73 ^A
	SW-T(N)	88.11 ± 2.70 ^B	98.85 ± 1.00 ^B
	SE-C(+E)	78.13 ± 1.22 ^A	80.86 ± 1.65 ^A
	SW-T(+E)	92.03 ± 2.78 ^B	100.56 ± 2.43 ^B

Values are represented as mean ± SE of three animals/group. Catalase activity is expressed as m mol of H₂O₂ degraded/min/mg protein. ABCD values with different superscripts between groups in each column are significantly different at *P* < 0.05; Animals of different ages except 12-mo-olds show significant regional variation. SE-C(N), sedentary control; SE-C(+E), sedentary control supplemented with vitamin E; SW-T(N), trained swimmers; SW-T(+E), trained swimmers supplemented with vitamin E; mos, months; LV, left ventricle; RV, right ventricle.

Table 2

Superoxide dismutase activity as a function of age, exercise and dietary vitamin E supplementation

Age (mos)	Groups	LV	RV
4	SE-C(N)	52.72 $\pm$ 4.56 ^A	63.10 $\pm$ 0.81 ^A
	SW-T(N)	101.98 $\pm$ 5.86 ^B	91.86 $\pm$ 5.24 ^B
	SE-C(+E)	78.84 $\pm$ 5.47 ^C	88.02 $\pm$ 7.25 ^C
	SW-T(+E)	109.54 $\pm$ 2.81 ^B	97.09 $\pm$ 3.60 ^B
8	SE-C(N)	71.47 $\pm$ 1.77 ^A	78.25 $\pm$ 0.77 ^A
	SW-T(N)	79.26 $\pm$ 1.84 ^A	78.98 $\pm$ 0.89 ^A
	SE-C(+E)	87.54 $\pm$ 3.89 ^B	85.64 $\pm$ 2.29 ^B
	SW-T(+E)	93.48 $\pm$ 2.36 ^C	91.41 $\pm$ 2.06 ^C
12	SE-C(N)	51.37 $\pm$ 0.84 ^A	57.19 $\pm$ 1.71 ^A
	SW-T(N)	59.01 $\pm$ 1.36 ^B	62.02 $\pm$ 1.90 ^B
	SE-C(+E)	85.15 $\pm$ 3.41 ^C	77.32 $\pm$ 0.92 ^C
	SW-T(+E)	86.90 $\pm$ 2.62 ^D	94.14 $\pm$ 2.54 ^D
22	SE-C(N)	53.65 $\pm$ 2.37 ^A	67.58 $\pm$ 1.98 ^A
	SW-T(N)	44.67 $\pm$ 2.28 ^B	57.48 $\pm$ 3.09 ^B
	SE-C(+E)	56.94 $\pm$ 1.81 ^A	70.30 $\pm$ 1.47 ^A
	SW-T(+E)	69.20 $\pm$ 1.36 ^C	85.54 $\pm$ 1.87 ^C

Values are represented as mean $\pm$ S.E of three animals/group. Superoxide dismutase activity is expressed as units/mg protein; ABCD values with different superscripts between groups in each column are significantly different at $P < 0.05$; Animals of different ages except 22-months do not show significant regional variation. SE-C(N), sedentary control; SE-C(+E), sedentary control supplemented with vitamin E; SW-T(N), trained swimmers; SW-T(+E), trained swimmers supplemented with vitamin E; mos, months; LV, left ventricle; RV, right ventricle.

a 27% higher enzyme activity was seen in the LV and RV of SW-T(+E) (Table 2).

3.1.1. Lipid peroxidation

A. MDA. MDA an index of LP was significantly low (3.5 fold) in the LV and RV (2.5 fold) of SW-T(+E) than SE-C(N) in the 4-mo-olds. Similarly, a two-fold decrease was observed in both the regions of SW-T(N). MDA content increased in the 8-mo-olds than the 4-mo-old animals. Although both the trained groups maintained a lower level of MDA, the extent of decrease in the 8-mo-olds was less than the 4-mo-olds. A 44% lower MDA content in the LV, a 34% decrease in the RV of SW-T(+E) followed by a 20% decrease in LV and a 25% lower MDA content in the RV of SW-T(N) over SE-C(N) was seen. However, in the 22-mo-olds, LV and RV of SW-T(+E) revealed no significant changes compared to SE-C(N) while a 13% higher MDA concentration was seen in SW-T(N) (Fig. 1).

B. LF-like autofluorescent material. 4- and 8-mo-old animals had lesser LF-like autofluorescent substances in the LV and RV of trainee groups as compared to their sedentary counterparts. Animals belonging to SE-C(+E) also showed a lower content. In the 12-mo-olds, SW-T(+E) had a 35% lesser accumulation while SW-T(N) was found to have a mere 11% low concentration of such substances. In the 22-mo-olds also, the vitamin E supplemented trained and sedentary groups were effective in restricting the accumulation of these LF-like autofluorescent substances in both

the regions than those animals which did not receive vitamin E supplementation. Accumulation was higher in the LV than the RV in this age group (Fig. 2).

Tissue vitamin E. Tissue vitamin E changes in the RV of 4-mo-olds showed an increase in all the three experimental groups over SE-C(N) with a greater increase in SW-T(+E). In the 8-, 12- and 22-mo-old animals, a reduction in tissue vitamin E was seen in the SW-T(N) group. Between the trained and sedentary groups supplemented with vitamin E, SW-T(+E) had a higher concentration of the supplemented vitamin in the 8- and 12-mo-olds, while in the 22-mo-olds, concentration of vitamin E significantly decreased (Fig. 3a).

Fig. 3b represents changes in tissue vitamin E in LV of trained and sedentary animals. Similar to RV, tissue vitamin E content decreased in 8-, 12- and 22-mo-old SW-T(N) groups. But an important feature in the LV of 22-mo-olds was a significant decrease in the vitamin E content even in the trained animals that received vitamin E.

The effects of age, exercise and AO on the above-mentioned parameters have been summarized in the Table 3.

4. Discussion

The changes in the anatomy of the heart that accompany normal aging may pose a varying scenario, when region-related adaptations to exercise and supplemented AOs are considered. An interpretation of our results on the defensive adaptations of the aging heart has to consider three factors, namely, (1) suitable swim training protocol as a possible means to evoke age-related positive responses in both the ventricles, (2) the influence of exercise alone on the aging heart and (3) a combination of exercise and vitamin E in evoking maximum responses in the AO system of the ventricles.

In the present investigation, CAT activity revealed an increase in all the age groups and this significant increase, indicated the efficient scavenging of excess H_2O_2 generated during exercise. Our data are consistent with the findings of previous reports indicating an increased CAT activity in tissues of rats subjected to treadmill exercise (Somani et al, 1995; Kim et al, 1996). Higher CAT activity was noticed in the LV of 4-, 8-, and 12-mo-olds, while in the 22-mo-olds, RV exhibited elevated activity. Young and middle-aged hearts failed to elicit a response of similar or greater magnitude than that of the old, although significant increase in CAT activity were evident when compared to their sedentary counterparts (Table 1). Thus exercise of the intensity and duration that has been applied in the present study seems to evoke higher enzyme activity even when initiated as late as 22-mos of age.

Young adults of 4 months exhibited remarkable increase in SOD activity in both the LV and RV, irrespective of vitamin E supplementation. The extent of increase was lower in the other age groups. SW-T(N) group failed to

Fig. 1. Myocardial lipid peroxidation (MDA) as a function of exercise and vitamin E supplementation in (a) 4-mo-olds, (b) 8-mo-olds, (c) 12-mo-olds and (d) 22-mo-olds. Values are means $\pm$ SE ($n = 3/\text{group}$). Significance between group means of four ages is analyzed by DMRT and represented in upper case at $p < 0.05$. Those not sharing the same letters are significantly different. 4- and 8-mo-olds do not show significant regional variation.

Fig. 2. Changes in the LF-like autofluorescent substances in the myocardium in relation to exercise and vitamin E supplementation in (a) 4-mo-olds, (b) 8-mo-olds, (c) 12-mo-olds and (d) 22-mo-olds. AF, autofluorescence. Values are mean $\pm$ SE ($n = 3/\text{group}$). Animals of all ages except 4-mo-old show significant regional variation.

Fig. 3. % Change in the vitamin E content as a function of age in the (a) RV and (b) LV of sedentary and trained rats supplemented with vitamin E when compared with their sedentary counterparts without vitamin E supplementation. Values are means $\pm$ SE ($n = 3$ /group).

show any increase in SOD in the 8-mo-olds, while in the 12-mo-olds, the extent of increase, though evident was to a much lesser degree than that seen in the 4-mo-olds. Although our assays are indicative of total SOD activity, it seems likely that the observed exercise-induction of SOD was due to an increase in the manganese isoform of SOD (Mn-SOD), since the cytosolic isoform (Cu–Zn SOD) is not inducible (Ji et al., 1988). The observed increase in cardiac SOD activity in the trained animals reflects the ability of the heart to scavenge excess superoxide radicals generated in the mitochondria in response to the oxidative stress. Furthermore, the enzyme activity was much higher in the LV than in the RV, thereby suggesting a better response of the LV to exercise, in 4- and 8-mo-old animals. The adaptive capacity, however seemed to decrease with age, as noted by the absence of regional variations in 12-mo-olds and a reversed SOD pattern of LV and RV in 22-mo-old animals, with the latter exhibiting a higher AOE activity suggesting a better free radical scavenging capacity in the RV. One possible mechanism for elevated AOE activities in

the trained animals could be that the mitochondria consumes more oxygen to provide ATP through oxidative phosphorylation. Adenosine, a product of ATP, is reported to have several regulative effects on the body's complex adaptation to exercise, one of which may be an induction of AOE activity (Simpson and Phillis, 1992). Furthermore, the fact that elevated activity was seen in the SW-T(N) rats prompts us to speculate that an AO supplementation may not be a pre-requisite in the young, unlike in the old, where an AO-enriched diet is essential. In the present study, the physical stress imposed was probably insufficient to evoke a positive response in the old animals, which could also point to a failing adaptive capacity in the old to challenge the oxidative stress and more so in animals that were deprived of vitamin E supplement.

Also supportive of the less beneficial role of physical exercise and vitamin E enriched diet in the 22-mo-old rats was the negligible variation in MDA between SW-T(N) and SW-T(+E) groups. An age-related increase in MDA content was noticeable, although the differences between 8- and 12-mo-old animals were insignificant. Trained and sedentary groups that received a daily dosage of vitamin E were more successful in curtailing LP, at least in the 4-, 8- and 12 mo-old animals. Training or a combination of training and vitamin E proved less beneficial in the old in terms of reducing LP. These findings may relate to the concept that vitamin E can inhibit LP and reduce the accumulation of LF, which is one of the products of LP (Marzabadi et al., 1991). These results are in accordance with our earlier in vitro studies on the primary cultures of myocytes as well as neurons, wherein it was demonstrated that the AO could substantially reduce the autofluorescent substances (Asha Devi et al., 1990; Kan et al., 1991). This conclusion not only points to the free-radical theory of aging but also suggests that aging in the heart is dependent on mechanisms that control the activity levels of various AO defense enzymes.

Our results on reduced tissue vitamin E in the unsupplemented animals of older ages may indicate the probable utilization of the AO during exercise. Depletion of tissue vitamin E in the 22-mo-old trained animals despite AO supplementation could underline the significance of the AO, which is extensively utilized by the animals in overcoming exercise-induced free radical damage. Perhaps a higher dose of vitamin E could have

Table 3
Three-way ANOVA analysis:

Parameters	Age	Groups	Regions	Age $\times$ Group	Age $\times$ Region	Group $\times$ Region	Age $\times$ Group $\times$ Region
SOD	S	S	S	S	S	S	S
CAT	S	S	S	S	S	S	S
MDA	S	S	NS	S	S	NS	NS
LF-like substances	S	NS	S	S	S	S	NS
Vitamin E	S	S	S	S	S	S	S

Three-way ANOVA (age, groups and regions), significant effects are noted by 'S' and those not significant as 'NS'.

proved beneficial. Also consumption of vitamin E, especially in SW-T(N) could be more necessary keeping in view the decline in AOE system. Vitamin E may be a possible non-enzymic AO to compensate for the lowered SOD activity in quenching singlet oxygen radicals. The failing AO system could be one of the causative factors for the extensive utilization of the supplied AO in eluding free radical-induced damage.

In conclusion, the AOE revealed a higher activity in the RV than in the LV thereby suggesting that although the adaptive response of the LV is efficient at younger ages, with advancing age, the compensatory capacity of the LV declines. This could be attributed either to its structural differences or to elevated oxidative stress resulting from increased load. Our studies are indicative of the relationship between AO micronutrients such as vitamin E and physical activity in reducing oxidative stress-related diseases in the old heart.

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