

Swim exercise training and adaptations in the antioxidant defense system of myocardium of old rats: relationship to swim intensity and duration

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Abstract

We examined a suitable swim program of different intensities and durations that could evoke changes in the myocardial antioxidant capacity in 22-month-old rats. Male rats (*Rattus norvegicus*) were assigned to either a sedentary control (SE-C) group or one of six trainee groups. Animals were swim-exercised for 4 weeks with either 20 min or 40 min/day, and three intensities, low, moderate and high. Low-intensity at 20 min/day elicited maximum swim velocity (S_v) and endurance capacity ($P < 0.05$). While serum total cholesterol, triglyceride and low-density lipoprotein (LDL-C) levels were significantly reduced, high-density lipoprotein (HDL-C) showed an increase ($P < 0.05$) in low-intensity trained rats (20 min/day) over SE-C. Notable reduction in blood lactate was also evident. Exercise training significantly increased superoxide dismutase (Mn-SOD), decreased lipid peroxidation products, malondialdehyde and lipofuscin in the left and right ventricles. Increased Mn-SOD with concomitant decrease in lipofuscin in left ventricle was significantly greater than in right ventricle. Moderate- to high-intensity exercise was not effective in either reducing lipid peroxidation products or elevating Mn-SOD activity. These data suggest that swim training at low-intensity of 20 min/day is beneficial as a major protective adaptation against oxidative stress in old myocardium.

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1. Introduction

One of several mechanisms affected in old age is the impairment of self-defense system against reactive oxygen species (ROS) related to an imbalance between the prooxidants and antioxidants (Somani et al., 1995). However, ROS injury is felt largely in tissues with post-mitotic cells (Miquel, 1998). Interestingly, the generation of ROS in old age resembles that of ROS-induced ischaemia-

reperfused (I-R) heart (Matheis et al., 1992). Alterations in DNA, protein and lipid oxidations result in an impairment of cardiac performance (Downey, 1990; Park et al., 1992). There have been an increasing number of studies that have tried to evaluate the role of endogenous as well as exogenous antioxidants in detoxifying ROS, a move to ameliorate age-related cardiac injury (Reznick et al., 1992; Powers et al., 1993; Anitha and Asha, 1996; Prathima and Asha, 1999). Of several methods to do so, physical training has proved to be a promising approach to boost the enzymic antioxidant system (AOS) in the heart

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Table 1
Training protocol showing exercise programme for 22-month-old male rats

Groups	Sub-groups	Pre-training (days)	Training			
			Intensity (% body mass)	Duration (min/day)	Frequency (days/week)	Total period (weeks)
I	IA					
	20L	4	2	20	5	4
	20M	4	3	20		
	20H	4	5	20		
II	IIA					
	40L	8	2	40	5	4
	40M	8	3	40		
	40H	8	5	40		

L, low; M, moderate; H, heavy; $n=4$ animals/sub-group.

IA, subgroups- 20L, 20M, 20H; IIA, subgroups- 40L, 40M and 40H.

even in the old (Asha et al., 2003b). However, it is essential to recognize the fact that physical exercise of an inappropriate nature in terms of duration and intensity may itself act as a powerful source of ROS.

Although exercise training has been suggested for young rodents on a motorized treadmill (Powers et al., 1993; Navarro-Arevalo et al., 1999), running wheel (Yamamoto et al., 2002) and swimming (Nakao et al., 2000), similar protocols are not evident for old rodents. Our earlier studies have indicated the benefits of swim training on the left and right ventricles (LV and RV) in old rat heart in terms of better adaptation to withstand the anaerobic conditions imposed by exercise (Prathima and Asha, 1999; Sharon and Asha, 2000). In old rats, firstly, there is an altered region-specific oxidative metabolism and, secondly, old hearts may suffer greater damage from ROS compared to younger ones due to reduced antioxidant defense capacity (Asha et al., 2003b).

In our studies, swimming has been chosen as a suitable model of endurance exercise training since swimming is a natural behavior of rodents (Kramer et al., 1993). In the present study, we have attempted to identify a suitable swim exercise protocol for old rats that can alter superoxide dismutase (Mn-SOD), lipid peroxidation (LP) and lipofuscin (LF) in the myocardium along with related changes in blood lipid profile.

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 albino Wistar rats of 4-month-old were obtained from the Central Animal Facility, IISc., Bangalore and maintained until they were 22-month-old in a clean rodent room. Animals were housed two per cage fitted with stainless-steel wire-mesh bottoms, at 28 ± 1 °C, in $77 \pm 4\%$ relative humidity and under a daily 12 h-light and 12 h-dark cycle. The animals were fed with lab chow (Lipton India Ltd, Bangalore) and tap water ad libitum.

2.2. Exercise training program

Table 1 denotes the categorization of rats into groups and subgroups. In brief, rats were randomly assigned to one of two groups-20 and 40 min, and three sub groups-low (2%), moderate (3%) or high (5%) depending on the intensity. Swim exercise training was similar to our earlier protocols with minor modifications (Asha et al., 2002). In brief, rats were made to exercise in groups of three in a glass tank ($77 \times 38 \times 39$ cm) filled with water to a height of 31 cm and at 32 ± 1 °C. Animals were trained daily between 10.00 h and 12.30 h. They were made to swim either with a load of 2, 3 or 5% of body mass for 20 or 40 min/d. The pre-training period lasted for 5 days in the 20 min group and 10 days in the 40 min group with 5 min of exercise/day. At the completion of exercise, rats were towel-dried and returned to their respective cages. No deaths occurred during or after exercise in any of the subgroups. Sedentary control group of rats were confined to cage activity and handled only during changing of cages.

2.3. Measurement of swim parameters

Swim velocity (S_v) as the distance traversed by the animals was measured in terms of m/min. External work done (W_{ext}) was calculated according to that described by Brooks and White (1978) and expressed as kg/min. Endurance capacities (E) of trainees were assessed at the conclusion of training period by allowing them to swim to exhaustion. Exhaustion is defined as the point (in min) where rats remained below the water surface for 10 s (Dawson et al., 1968).

2.4. Blood and serum analyses

Animals were lightly anaesthetized with ether and restrained in dorsal recumbency and blood was collected from the tail in EDTA-containing tubes and centrifuged to obtain the plasma for lactate estimation. For serum analyses, blood was drawn from a syringe needle inserted just below the xyphoid cartilage and slightly to the left of midline (Asha et al., 2003a). Fresh clear serum was separated from the clotted blood by centrifuging the samples at 1300 g for 5 min.

2.4.1. High-density lipoprotein-cholesterol (HDL-C)

The HDL-C reagent provided in the kit (Rashmi Diagnostics, Bangalore, India) containing $MgCl_2$ was added to serum, mixed and left for 10 min at 25 °C to allow the precipitation of chylomicrons, low density lipoproteins (LDL) and very low density lipoproteins (VLDL). Samples were centrifuged at 2600 g for 15 min. To the supernatant containing HDL-C, cholesterol reagent was added, mixed and incubated for 10 min at 37 °C. Absorbance was read at 505 nm in a spectrophotometer (Systronics, Model 115, India) and HDL-C was expressed as milligram per litre.

2.4.2. Low-density lipoprotein-cholesterol (LDL-C)

LDL-C in the serum was precipitated by addition of LDL reagent in the kit (Rashmi Diagnostics), vortex mixed and centrifuged at 2600 g for 10 min. Cholesterol in the supernatant was assayed by cholesterol reagent and the absorbance was measured at 505 nm. Concentration of LDL-C was obtained from the difference between total cholesterol and cholesterol in the supernatant and was expressed as milligram per litre.

2.4.3. Total cholesterol (TC)

Serum was incubated with the cholesterol reagent (Rashmi Diagnostics) for 10 min at 37 °C and absorbance measured at 505 nm and expressed as milligram per litre.

2.4.4. Triglycerides (TG)

Serum was treated with the triglyceride reagent (Rashmi Diagnostics), mixed, incubated for 10 min at room temperature (RT) and absorbance read at 505 nm. TG content was expressed as milligram per litre.

2.4.5. Plasma lactate

Lactate was measured by the method of Barker and Summerson (1965). Glucose and other interfering materials of the protein-free filtrate were removed and an aliquot of this was heated with concentrated sulfuric acid. The resulting aldehyde was reacted with *p*-hydroxyphenol and copper ions, and absorbance measured at 560 nm using lithium lactate as standard. Lactate content was expressed as millimolar per litre.

2.5. Preparation of tissues

The abdomen and chest were opened by a midline incision and the heart was perfused with ice-cold buffered saline. The organ was excised, trimmed of blood vessels and connective tissue. Total organ and regional masses were recorded and processed for the following analyses.

2.5.1. Sub cellular fractionation of superoxide dismutase (SOD, EC.1.15.1.1)

Heart samples were rapidly homogenized in 3 vol (w/v) of cold homogenizing buffer containing 0.25 M sucrose, 10 mM Tris-Cl and 1 mM EDTA (pH 7.4), and processed further for obtaining the mitochondrial fraction (Bruce et al., 1984). In brief, the homogenate was diluted suitably and centrifuged at 300 g for 15 min at 4 °C. The '10K' pellet was diluted with 10 mM Tris-Cl, (pH 7.4) mixed with 10% SDS and incubated at 37 °C for 30 min and cooled to 4 °C. The resulting sample was centrifuged at 20 000 g for 10 min. The supernatant was used for SOD assay by the ferricytochrome C reduction method (Flohe and Otting, 1984) in the presence of 2 mM cyanide at 550 nm.

Table 2

Body mass as a function of exercise intensity and duration

Age (months)	Weeks of training	20L	20M	20H	40L	40M	40H	SE-C
		A	B	B	B	B	B	C
22	1	498 ± 1.85 ^a	497 ± 1.76	498 ± 2.51	497 ± 1.52	497 ± 1.45	498 ± 1.2	501 ± 0.88
	2	501 ± 1.76 ^b	499 ± 1.45	500 ± 2.51	498 ± 1.76	499 ± 2.44	500 ± 1.0	505 ± 0.88
	3	504 ± 1.45 ^c	502 ± 1.45	501 ± 2.66	500 ± 1.76	500 ± 1.20	501 ± 1.15	509 ± 1.00
	4	508 ± 1.20 ^d	504 ± 1.52	503 ± 2.72	501 ± 0.57	501 ± 1.20	501 ± 1.15	511 ± 1.20

Body mass (g) showed significant differences between weeks.

Significance between group means of different intensities and durations as analysed by two-way ANOVA followed by Duncan's multiple range test is significant at $P < 0.05$ and is represented by the uppercase (A, B and C) and between weeks in lower case (a, b, c and d). Those not sharing the same letters are significantly different.

SE-C, Sedentary control.

L, low; M, moderate; H, heavy; $n = 4$ animals/sub-group.

2.5.2. Protein determination

Protein was determined by the method of Lowry et al. (1951) using bovine serum albumin as the standard.

2.5.3. Extraction and quantification of lipofuscin (LF)

Heart samples were homogenized in 2:1 chloroform: methanol at room temperature (RT) for 10 to 15 mins. Extraction of LF was by the method of Csallany and Ayaz, (1975), 0.5 ml was chromatographed on Sephadex LH-20 columns and 0.1 ml aliquots were collected. Fluorescence was measured at an excitation wavelength of 365 nm and emission wavelength of 435 nm (Genway, Model 6200, UK). The equipment was calibrated with 0.1 N quinine sulfate to read 100 units and fluorescence was represented as units/100 mg tissue.

2.5.4. Lipid peroxidation (LP)

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

2.6. Statistical analyses

All data were expressed as mean ± S.E. SOD, LF and MDA were analyzed within a two-factor analysis of variance (ANOVA) between weeks and groups. Plasma lactate and serum lipid profile were analyzed by one-factorial ANOVA. When a significant F ratio was found, Duncan's multiple range tests (DMRT) were used to assess the differences between group means. Paired t -test was performed to analyze the significance of Total cholesterol/

HDL-C level between the sedentary controls and trainees. Probability values (P) < 0.05 were considered significant (Snedecor and Cochran, 1994).

3. Results

3.1. Body mass

There was significant reduction in body mass between the different subgroups with respect to the sedentary controls at the end of each week. Changes in body mass were insignificant between the low- to moderate-intensity exercise and between moderate-to high-intensity in rats trained to swim for 40 and 20 min/d. No changes were seen during the third and fourth week of training in all subgroups (Table 2).

3.2. Effect of exercise on swim parameters

Final S_v values showed significant reductions between all groups except between 20H and 40M subgroups. Maximum and minimum velocities were significantly different in rats of 20L and 40H subgroups (Fig. 1a). W_{ext} increased linearly with increased load of 2 to 5% body mass. In rats that swam for 20 min, the changes were significant only between those carrying loads of 2 and 5% body mass (Fig. 1b). Better endurance capacity was evident in animals trained for low intensity and for a duration of 20 min/d. Endurance decreased with increasing intensity and duration. Changes that were seen between low-, moderate- and high-intensity exercise were significant in rats allowed to swim for 20 min, but not between low- to moderate-exercised rats of 40 min duration (Fig. 1c).

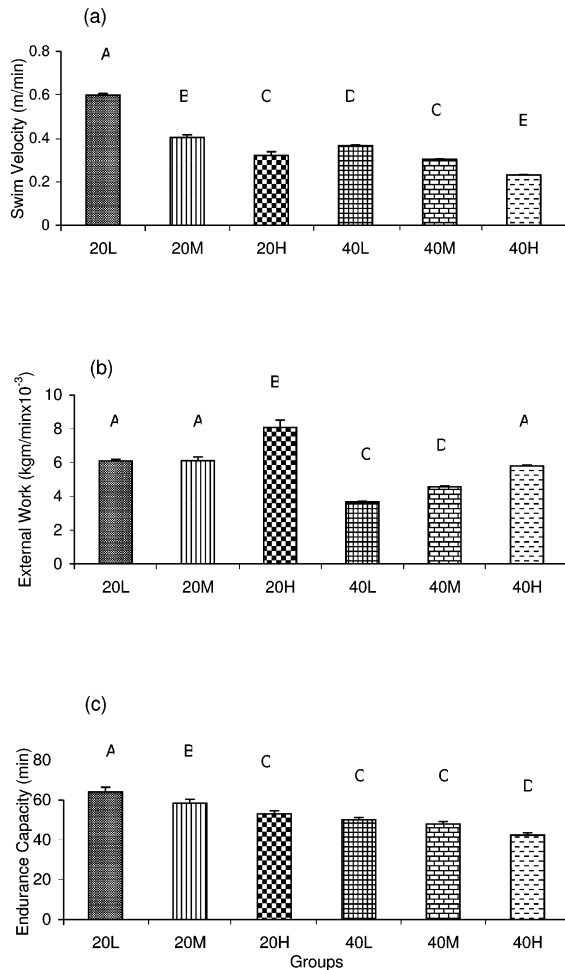


Fig. 1. Swim parameters in relation to exercise duration and intensity in 22-month-old male rats. (a) Swim velocity (S_v), (b) External work done (W_{ext}), (c) Endurance (E). Significance between the group means of different intensities and durations as analyzed by one-way ANOVA followed by Duncan's multiple range test is significant at $P < 0.05$ and is represented by the upper case. Those not sharing the same letters are significantly different. Values are mean \pm S.E. of four animals/group.

3.3. Effect of exercise on serum lipid profile and blood lactate

Significant reductions in total cholesterol were seen between the animals trained for 2 and 3% body mass (11.2 and 6.3% over the SE-C) and between 2 and 5% body mass (5.1 and 0.67% over the SE-C) in those trained to swim for 20 min and 40 min, respectively (Fig. 2a).

HDL-C increased significantly over the SE-C and was greater in the 20 min group and the extent

of changes were smaller in the 40M and 40H (15 and 11%) compared to their 20 min counterparts (44.2 in 20M and 24% in 20H) (Fig. 2b).

LDL-C levels were reduced significantly in rats that swam at low- and moderate-intensity of 20 min/d (31% and 24%, respectively) as well as for 40 min/d (26 and 15% at low- and moderate-intensity) with significant changes between the subgroups (Fig. 2c).

TG was also reduced in the serum of rats trained for 20 min/d (24, 19 and 12% in low-, moderate- and high-intensity) as well as 40 min/d (18 and 13% at low- and moderate-intensity) (Fig. 2d).

Total cholesterol/HDL-C ratios decreased significantly in animals trained for 20 min/d (44, 35 and 20% at low-, moderate- and high-intensity) and 40 min/d (35 and 16% at low- and moderate-intensity) (Table 3).

Blood lactate was reduced significantly between rats that swam at low-intensity (71%) and moderate-intensity (29%) of 20 min/d. High-intensity exercise of 20 min/d resulted in an increase in lactate (70%) and this trend was also noticeable in rats trained for 40 min/d where increases of 11.5%, 42.5% and 82.5% were seen in low-, moderate- and high-intensity exercised rats, respectively (Fig. 3).

3.4. SOD activity

Mn-SOD activity was significantly higher in the left ventricle than in the right ventricle in all groups. Low- and moderate-intensity exercise at 20 min/d significantly elevated Mn-SOD activity by 42.4% and 10.5%, respectively, in the left ventricle. In the right ventricle, low-, moderate- as well as high-intensity exercise increased the enzyme activity by 33, 25 and 22% over the controls. Low-intensity exercise of 40 min/d also resulted in an increase of Mn-SOD activity by 25% of the control level (Fig. 4).

3.5. Lipid peroxidation

Myocardial MDA content decreased significantly with training in all groups over the sedentary controls. Decreases were by 49, 48, and 43.5% in rats trained 20 min/day and by 47.5, 47 and 32% over the sedentary controls in those trained for 40 min/day at low-, moderate- and high-intensity. However, no changes were noted between the trained groups except the high-intensity of 40 min/

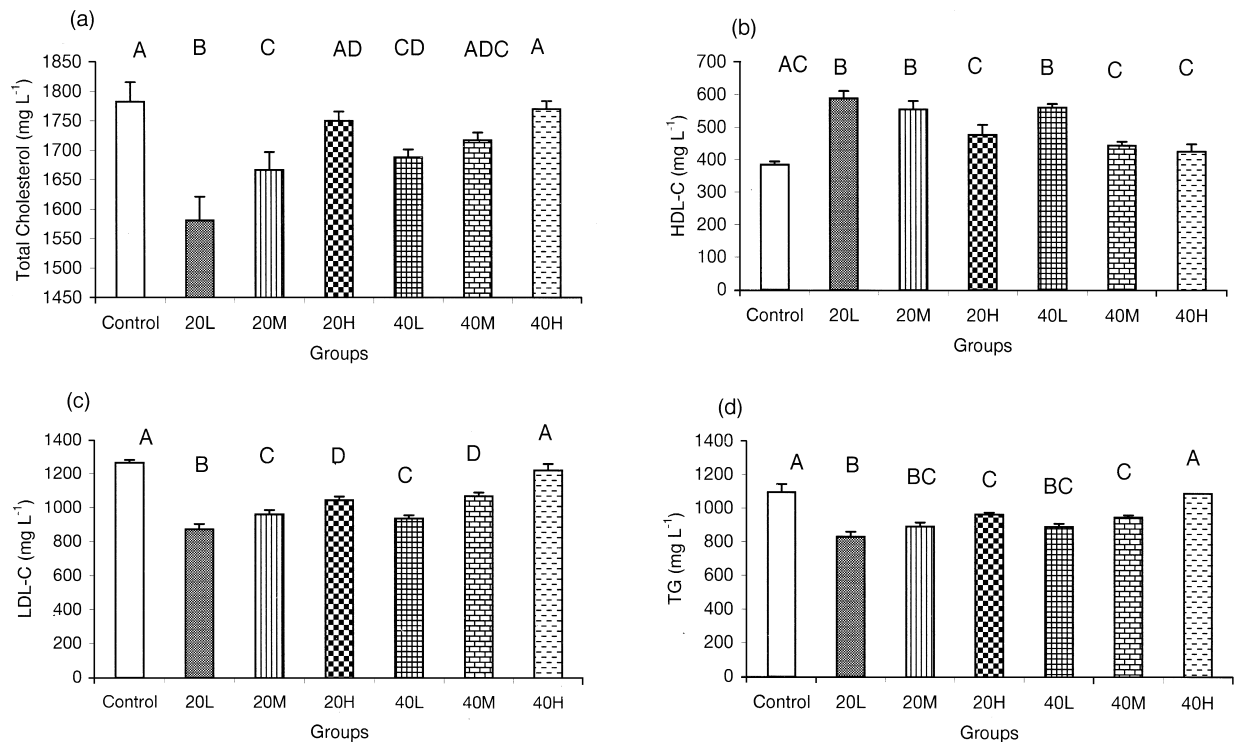


Fig. 2. Serum lipid profile in animals subjected to different intensities and durations of swim exercise. (a) Total cholesterol (b) HDL-C (c) LDL-C and (d) TG. Significance between the group means of intensities and durations as analyzed by Duncan's multiple range test at $P < 0.05$ and is represented in upper case. Those not sharing the same letters are significantly different. Values are mean \pm S.E. of four animals/group.

d. Changes were not significant between the regions (Fig. 5a).

3.6. Lipofuscin

Training brought about a significant decrease in lipofuscin content of left as well as right ventricle. Rats trained to swim at low-, moderate- and high-

intensity showed depletions by 27, 36 and 21% (20 min/d) and by 34.5, 10, and 1% (40 min/d) in the left ventricle while depletions were of 17, 6.5 and 17% (20 min/d) and by 21, 8 and 4% (40 min/d) above the SE-C in the right ventricle

Table 3
Effect of intensity and duration of exercise on serum Total cholesterol/HDL-C

Groups	Total cholesterol/HDL-C
Sedentary control	4.61 \pm 0.04
20L	2.68 \pm 0.039* *
20M	3.01 \pm 0.08* *
20H	3.69 \pm 0.21* *
40L	3.00 \pm 0.03* *
40M	3.86 \pm 0.08*
40H	4.16 \pm 0.23

The values are mean \pm S.E. of four animals/sub-group. Differences were evaluated between the sub-groups and sedentary control through paired *t*-test. * $P < 0.05$; * * $P < 0.001$.

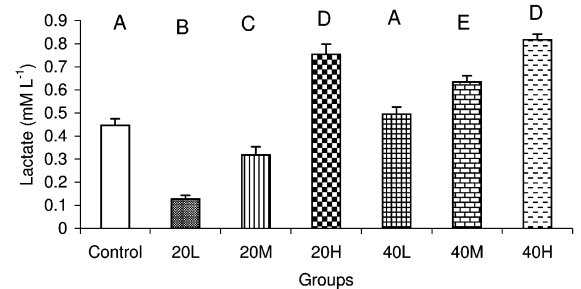


Fig. 3. Plasma lactate in animals subjected to different intensities and durations of swim exercise. Significance between the group means of four ages as analyzed by Duncan's multiple range test is at $P < 0.05$ and is represented in upper case. Those not sharing the same letters are significantly different. Values are mean \pm S.E. of four animals/group.

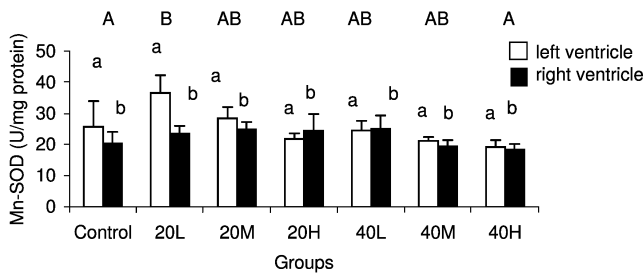


Fig. 4. Myocardial Mn-SOD activity in the left (open bars) and right ventricle (filled bars) of animals subjected to different intensities and durations of swim exercise. Significance between group means of intensities and durations as analyzed by Duncan's multiple range test is at $P < 0.05$ and is represented in upper case and between the left and right ventricle in lower case. Those not sharing the same letters are significantly different. Values are mean \pm S.E. of four animals/group.

myocardium. No changes were seen between different intensities of 20 and 40 min/d except the low- and moderate-intensity, trained for 40 min/d (Fig. 5b).

4. Discussion

The free radical theory shares a common feature with aging and exercise, i.e. induction of oxidative

stress. There appeared to be a definite relationship between body masses of sedentary and swim trained animals in that the trainees were less heavy than the less active sedentaries. However, the reduction in body mass was significant between rats trained at low- and moderate- intensity for 20 min/d during all the four weeks of training regime, but insignificant between all other intensities and durations. Therefore low- and moderate-intensity of 20 min/d and 40 min/d, respectively, are beneficial in reducing the body mass in the old rats.

Studies from our laboratory on rats have indicated greater biochemical adaptability in swimmers than voluntary runners (Bhagavathi and Asha, 1993) when exercise was initiated at 4- (young) or 12- (middle-age) months of age. Swim training has been used as a suitable method of endurance training because of the innumerable benefits derived out of it over treadmill running. A few of these include possible difference in the sympathoadrenal function between swimming and running (Geenen et al., 1988), less mechanical stress and injury due to buoyancy and reduced effects of gravity, as well as better redistribution of blood

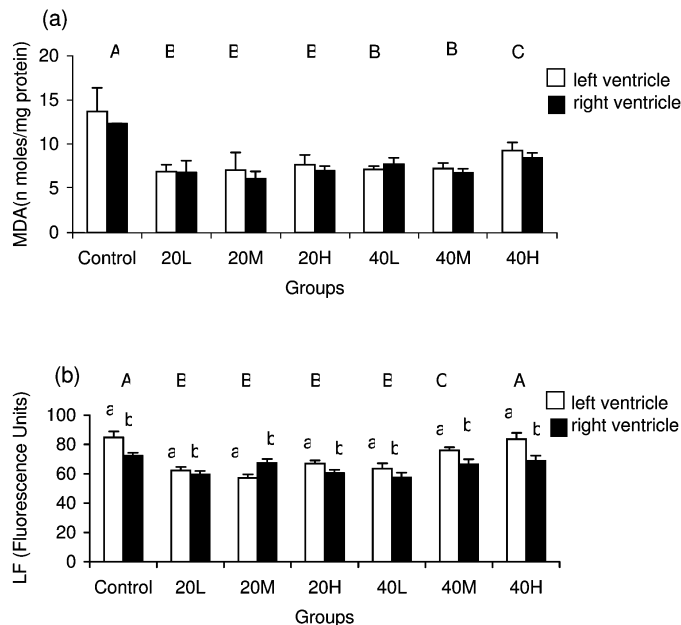


Fig. 5. Myocardial lipid peroxidation products- (a) MDA and (b) LF, in the left (open bars) and right ventricle (filled bars) of animals swim-trained at different intensities and durations. Significance between group means of exercise intensities and durations as analyzed by two-way ANOVA (regions and sub-groups) followed by Duncan's multiple range test is significant at $P < 0.05$ and is represented in upper case and between the regions in lower case. Those not sharing the same letters are significantly different. Values are mean \pm S.E. of four animals/group.

flow among tissues without significant variations in cardiac output and heart rate which in turn may minimize the magnitude of injury caused due to the generation of ROS.

Training-induced increase in swim velocity and endurance capacity at low-intensity of short duration was evident in the current study. The external work done decreased with duration and increased with intensity and, was inversely related to swim velocity. In an earlier study, we suggested that the enhanced swim performance capacity when assessed through better S_v and W_{ext} in 22-month-old rats that swam for 30 min/d at moderate-intensity is due to exercise per se ruling out the possible role of supplemental antioxidants such as vitamin E as an ergogenic aid in enhancing performance capacity (Asha et al., 2003a). Perhaps, the low endurance in rats seen at moderate- and high-intensities of 40 min/d may be improved by supplementation of an antioxidant such as vitamin E. A reduction in skeletal muscle mass and reduced physical performance capacity in old age may be related to decreased protein synthesis. Besides, deficiency of vitamin E has been reported to increase the susceptibility to free radical damage in exercised rats leading to premature exhaustion and reduction in endurance capacity (Davies et al., 1982), and these may have a greater implication in the old. Hence, our results suggest that swim training at low-intensity of 20 min/d in the old can probably reduce muscle loss and increase performance capacity. Further, rats trained to swim at low-intensity of 20 min/d exhibited the lowest level of plasma lactate compared with other protocols, wherein maximal lactate steady state indicates the higher blood lactate concentration where lactate removal capacity compensates its entry (Mader and Heck, 1986; Poole et al., 1988). Our data suggest that old SE-C are able to keep a stable blood entry or removal ratio in workloads upto 3% of their body mass when trained to swim for 20 min/d. At higher loads, blood lactate increases thereby suggesting higher production in relation to removal. Such information permits us to postulate that for old animals loads below 3%, considered to be 'sub-threshold', are the threshold that must be achieved if trying to improve their endurance capacity. This sub-threshold may also be indicative of an increase in anaerobic threshold along with the duration of training.

Our swim training program provided an initial pre-training period whose duration was gradually

lengthened to facilitate stress-free swimming. This stabilized blood lactate is probably due to muscle aerobic adaptations leading to lower lactate production for the same duration, but for higher intensities or increased blood lactate removal (Gladden, 2000; Donovan and Pagliassotti, 2000). The present finding of significantly reduced serum cholesterol at low and moderate-intensity of 20 min/d and at low-intensity of 40 min/d are comparable to those reported by Jeff et al. (1999) where rats swam 70 min/d, four times/week, for 21 weeks, without any load. Others have reported reduction in LDL-C levels in rats that swam for 2–6 h/day, 5 days/week, for 5 weeks (Ghaemmaghami et al., 1986). Our earlier studies on 22-month-old male rats that swam with moderate-intensity for 30 min/d, 5 days/week, for 4 weeks indicated no reductions in plasma total cholesterol and LDL-C while HDL-C increased (Asha et al., 2003a). The link between heart disease and oxidants has been established in relation to LDL-C, which promotes the formation and deposition of foam cells and subsequent evolution of atherosclerotic plaques (Steinberg, 1990). Although exercise HDL-C levels are inversely related to coronary heart disease (CHD) risks, the ratio of total cholesterol: HDL-C is considered a better marker (Willett, 1994). Our results have indicated reductions in the ratio in animals trained at different intensities and different durations with the low-intensity group of 20 min/d exhibiting maximal decrease of 44% over the sedentary.

Physical exercise can increase ROS production in the heart (Ji, 1994; Bejma et al., 2000) since O_2 consumption increases ROS at the electron transport chain, although it may account for only 2–5% of the total oxygen (Tiidus and Houston, 1995). In addition to training intensity and duration, another important parameter that we have addressed in this study is to elucidate the regional responses, but not the whole heart, to exercise. Since the left ventricular wall is thicker than that of the right it varies also in its anatomical and functional components and it has been reviewed recently in relation to myocardial aging (Asha, 2002). Mn-SOD activity was significantly higher in the left ventricle compared to the right ventricle. Our results have indicated an increase in Mn-SOD activity in old animals trained at low-intensity for 20 min/d. Exercise of higher intensities and durations failed to elevate Mn-SOD activity in either the LV or the RV. Our speculation is that intensive

exercise, which can increase O₂ consumption by almost 10-fold, can in fact harm the ventricles due to an unproportional generation of super oxide radicals leading to free radical injury (Meydani, 1995). Our results are similar to those of Navarro-Arevalo et al. (1999) on young (3–5 months) and old animals (24–27 months) subjected to treadmill running, wherein old animals do not induce SOD, which relates to our recent findings on swim trained old rats (Asha et al., 2003b). Studies have indicated that exercise training can reduce heart vitamin E concentration (Tiidus and Houston, 1994; Asha et al., 2003b), and age-dependent dietary supplementation of vitamin E might protect against moderate- and high-intensity exercise-induced ROS. It would be interesting to examine other enzymic antioxidants such as glutathione peroxidase and catalase which may function as better free radical scavengers in the old since an earlier study indicated elevated catalase in the myocardium of left ventricle as well as right ventricle when rats were swim trained at moderate-intensity of 30 min/d for 4 weeks (Asha et al., 2003b).

In the current study rats trained at low- to moderate-intensity of 20 and 40 min/d showed a decline in lipid peroxidation in terms of MDA content. Increase in Mn-SOD activity was accompanied by a corresponding decrease in MDA of left (49%) and right ventricle (45%) in the low-intensity (20 min/d) trained rats. Despite the fact that there was no increase in the Mn-SOD activity at moderate- to high-intensity of 20 and 40 min/d, MDA was reduced significantly over the controls in these animals. Lipofuscin is one of the products that decreased to a greater extent in the left ventricle than the right at all intensities except at high-intensity, 40 min/d.

In summary, the present study demonstrates that endurance training at low-intensity of 20 min/d alters the myocardial Mn-SOD activity and lipid peroxidation products to different extents in the left and right ventricle of 22-month-old rats. Our data also indicate that this intensity and duration are effective in altering the serum lipid profile. The study demonstrates that the frequency, duration and intensity of swim training are parameters that may have to be critically considered while evaluating the benefits of swim training. However, the observed training protocol needs further studies in relation to gender.

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References

- Anitha, V., Asha, D.S., 1996. Age-related responses of right ventricle in swim-trained rats: changes in lactate and pyruvate contents and lactate dehydrogenase activity. *Mech. Ageing Dev.* 90, 72–75.
- Asha, D.S., 2002. Studies of the effects of exercise and vitamin E on the heart in animal models of aging. *Ind. J. Gerontol.* 16, 79–95.
- Asha, D.S., Jolitha, A.B., Prathima, S., 2002. Temperature-induced changes in anaerobic capacity of interventricular septum in trained young and middle-aged rats. *Biogerontology* 3, 155–162.
- Asha, D.S., Prathima, S., Subramanyam, M.V.V., 2003a. Dietary vitamin E and physical exercise: I. Altered endurance capacity and plasma lipid profile in ageing rats. *Exp. Gerontol.* 38, 285–290.
- Asha, D.S., Prathima, S., Subramanyam, M.V.V., 2003b. Dietary vitamin E and physical exercise: II. Antioxidant status and lipofuscin-like substances in aging rat heart. *Exp. Gerontol.* 38, 291–297.
- Barker, , Summerson, B.L., 1965. Lactic acid. In: Oser, B.L. (Ed.), *Hawk's Physiological Chemistry*. Tata McGraw-Hill, New Delhi, pp. 1127.
- Bejma, J., Ramires, P., Ji, L.L., 2000. Free radical generation and oxidative stress with ageing and exercise: differential effects in the myocardium and liver. *Acta Physiol. Scand.* 169, 343–351.
- Bhagavathi, R., Asha, D.S., 1993. Interaction of exercise and age on substrates of carbohydrate metabolism. *Ind. J. Exp. Biol.* 31, 72–75.
- Brooks, G.A., White, T.P., 1978. Determination of metabolic and heart rate responses of treadmill exercise. *J. Appl. Physiol.* 45, 1009–1015.
- Bruce, , Geller, L., Winge, D.R., 1984. Subcellular distribution of superoxide dismutases in rat liver. *Method Enzymol.* 105, 105–108.
- Csallany, A.S., Ayaz, L.L., 1975. Quantitative determination of organic solvent soluble lipofuscin pigments in tissues. *Lipids* 2, 412–417.
- Davies, K.J.A., Quintanilha, A.T., Brooks, G.A., Packer, L., 1982. Free radicals and tissue damage produced by exercise. *Biochem. Biophys. Res. Commun.* 107, 1198–1205.
- Dawson, C.A., Nadel, E., Hovarth, S.M., 1968. Cardiac output in the cold stressed swimming rats. *Am. J. Physiol.* 214, 320–325.
- Donovan, C.M., Pagliassotti, M.J., 2000. Quantitative assessment of pathways for lactate disposal in skeletal muscle fiber types. *Med. Sci. Sports Exercise* 32, 772–777.

- Downey, J., 1990. Free radicals and their involvement during long-term myocardial ischemia and reperfusion. *Annu. Rev. Physiol.* 52, 487–504.
- Flohe, L., Otting, F., 1984. Superoxide dismutase assays. *Method Enzymol.* 105, 93–104.
- Geenen, D., Buttrick, P., Scheuer, J., 1988. Cardiovascular and hormonal responses to swimming and running in the rat. *J. Appl. Physiol.* 65, 116–123.
- Ghaemmamghami, F., Sassolas, A., Gauguelin, G., Favier, R., Vincent, M., Sassard, J., et al., 1986. Swim training on genetically hypertensive rats of the Lyon strain: effects of plasma lipids and lipoproteins. *J. Hypertension* 4, 319–324.
- Gladden, L.B., 2000. Muscle as a consumer of lactate. *Med. Sci. Sports Exercise* 32, 764–771.
- Jeff, C., Volkan, S., Don, K., Marjorie, A.J., 1999. Effects of exercise detraining on lipid storage in rats. *Trans. Illinois State Acad. Sci.* 92, 203–209.
- Ji, L.L., 1994. Exercise-induced oxidative stress in the heart. In: Sen, C.K., Packer, L., Hanninen, O (Eds.), *Exercise and Oxygen Toxicity*. Elsevier, Amsterdam, pp. 249–268.
- Kramer, K., Dijkstra, H., Bast, A., 1993. Control of physical exercise of rats in a swimming basin. *Physiol. Behav.* 53, 271–276.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Measurements with the Folin-phenol reagent. *J. Biol. Chem.* 193, 265–275.
- Mader, A., Heck, H., 1986. A theory of the metabolic origin of anaerobic threshold. *Int. J. Sports Med.* 7, 45–65.
- Matheis, G., Sherman, M.P., Buckberg, G.D., Haybron, D.M., Young, H.H., Ignarro, L.J., 1992. Role of L-arginine-nitric oxide pathway in myocardial reoxygenation injury. *Am. J. Physiol.* 162, H616–H620.
- Meydani, M., 1995. Dietary vitamin E in oxidative stress and aging. In: Cutler, R.G., Packer, L., Berytram, J., Mori, A (Eds.), *Oxidative Stress and Aging*. Birkhäuser Verlag, Boston, pp. 331–343.
- Miquel, J., 1998. An update on the oxidative stress-mitochondrial mutation theory of aging: genetic and evolutionary implications. *Exp. Gerontol.* 33, 113–126.
- Nakao, C., Ookawara, T., Kizaki, T., Oh-ishi, S., Miyazaki, H., Haga, S., et al., 2000. Effects of swimming training on three superoxide dismutase isoenzymes in mouse tissues. *J. Appl. Physiol.* 88, 649–654.
- Navarro-Arevalo, A., Canavate, C., Sanchez-del-Pino, M.J., 1999. Myocardial and skeletal muscle aging and changes in oxidative stress in relationship to rigorous exercise training. *Mech. Ageing Dev.* 108, 207–217.
- Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* 95, 351–358.
- Park, Y., Kenekal, S., Kehrer, J.P., 1992. Oxidative changes in hypoxic rat heart tissue. *Am. J. Physiol.* 260, H1395–H1405.
- Poole, D.C., Ward, S.A., Gardner, G.W., Whipp, B.J., 1988. Metabolic and respiratory profile of the upper limit for prolonged exercise in man. *Ergonomics* 31, 1265–1289.
- Powers, S.K., Criswell, D., Lawler, J., Martin, D., Lieu, F.K., Ji, L.L., et al., 1993. Rigorous training increases superoxide dismutase activity in ventricular myocardium. *Am. J. Physiol.* 265, H2095–H2098.
- Prathima, S., Asha, D.S., 1999. Adaptations in lactate dehydrogenase and its isoenzymes in aging mammalian myocardium: Interaction of exercise and temperature. *Mech. Ageing Dev.* 108, 61–75.
- Reznick, A.Z., Witt, E., Matsumoto, M., Packer, L., 1992. Vitamin E inhibits protein oxidation in skeletal muscle of resting and exercised rats. *Biochem. Biophys. Res. Commun.* 189, 801–806.
- Sharon, C., Asha, D.S., 2000. Myosin ATPase in young and old mammalian myocardium: responses to exercise in cold and thermoneutral environment. *Ind. J. Exp. Biol.* 39, 29–34.
- Snedecor, G.W., Cochran, W.G., 1994. *Statistical Methods*. Iowa State Univ. Press, Ames, IA.
- Somani, S.M., Frank, S., Rybak, L.P., 1995. Responses of antioxidant system to acute and trained exercise in rat heart subcellular fractions. *Pharmacol. Biochem. Behav.* 51, 627–634.
- Steinberg, D., 1990. Lipoproteins and atherogenesis. *Current concepts*. *J. Am. Med. Assoc.* 264, 3047–3052.
- Tiitus, P.M., Houston, M.E., 1994. Antioxidant and oxidative enzyme adaptations to vitamin E deprivation and training. *Med. Sci. Sports. Exercise* 26, 354–359.
- Tiitus, P.M., Houston, M.E., 1995. Vitamin E status and response to exercise training. *Sports Med.* 20, 12–23.
- Willett, W.C., 1994. Diet and health. What should we eat? *Science* 264, 532–537.
- Yamamoto, T., Ohkuwa, T., Itoh, H., Sato, Y., Naoi, M., 2002. Effect of gender differences and voluntary exercise on antioxidant capacity in rats. *Comp. Physiol. Biochem. C* 132, 437–444.