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Original Article

Effect of Moderate Aerobic Exercise Training on Autonomic Functions and its Correlation with the Antioxidant Status

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Abstract

Aerobic exercise is a proven measure to enhance the cardio-respiratory efficiency. This improvement is primarily due to improvement in autonomic function where in there is increase in the parasympathetic function and decrease in sympathetic function. Exercise also affects many metabolic activities in our body and may be one of the factors which reduce the oxidative stress. The aim of the present study was to see the effect of moderate aerobic exercise training on autonomic function and its correlation with antioxidant status. 30 healthy volunteers in the age group of 18-22 years were screened. Autonomic function tests included activity (tone) and reactivity parameters. Antioxidant status was assessed by the level of malondialdehyde in plasma. We observed a significant change in SBP, DBP, LF nu, HF nu, delta value of DBP in CPT and MDA.

Our findings are consistent with earlier findings that short duration physical training is known to reduce blood pressure and that there is a relationship between HF (in HRV) and training response Physical exercise also provides a favorable change in the biochemical parameters such as MDA.

Introduction

In the modern electronic age, keeping fit has become a primary concern for people of all ages. Medical science tries to achieve an optimum physical and mental health of the individual through preventive, curative and promotive means.

Aerobic exercise is an exercise that involves or improves oxygen consumption by the body. These exercises can be performed at moderate level of intensity for extended period of time. Both the term

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and the specific exercise method were developed by Kenneth H. Coopper and Col. Pauline Potts.

Aerobic exercise also works to increase the mechanical efficiency of the heart by increasing cardiac output which is proportionate to increase in oxygen consumption (1). It also increases total pulmonary ventilation (2).

Autonomic functions are the involuntary vital functions regulated by Autonomic Nervous System. The Autonomic Nervous System is part of the peripheral nervous system that controls the visceral functions. It is divided into parasympathetic nervous system and sympathetic nervous system. Physical exercise training leads to physiological adaption of cardiovascular system by decreasing the sympathetic responses and increasing the parasympathetic responses (3, 4, 5, 6).

Antioxidants are substances that are capable of counteracting the damage done by the process of oxidation in our body. Free radicals such as superoxide anion radical, H_2O_2 and OH^- are common product in an aerobic environment and these agents are responsible for oxygen toxicity. To survive living organism generate a variety of water and lipid soluble antioxidant compounds. Antioxidants include nutrients (vitamins & minerals) as well as enzymes, their role are to neutralize excess free radicals.

Exercise is considered as an acceptable method for improving and maintaining physical and emotional health (4). The present study was planned to study the effect of aerobic exercise training on autonomic function and its correlation with antioxidant status.

Material and Methods

The present study was carried out in the department of physiology, Subharti Medical College, Meerut. 30 healthy volunteers were selected for the study. Volunteers in the age group of 18-22 years were screened and detailed medical history was taken to exclude any morbid state, which can influence the autonomic responses or poses a contraindication for exercise training. The physiological and biochemical assessment of autonomic function and antioxidant status was done by a battery of tests. The protocol of the study was approved by the institutional ethics committee. All subjects underwent the following tests before and after the exercise for the assessment of autonomic function and antioxidant status

Autonomic function tests

Autonomic parameters are divided into sympathetic and parasympathetic responses with activity (tone) and reactivity parameters.

Autonomic activity was assessed by performing heart rate variability (HRV) which quantifies autonomic drive to the myocardium and resting BP.

Sympathetic reactivity parameters included HGT, CPT and LST while parasympathetic reactivity parameters included Valsalva Ratio and Expiration: Inspiration ratio.

Prior instructions were given to the subjects before recording that they should not :

- Consume any medicine 24 hrs before recording.
- Consume tea, coffee, other caffeinated beverages such as coke etc and heavy meals at least 2 hours before the recording.

The subjects were given proper instruction about the test and recording procedure before starting the test and were made to relax and be comfortable.

Basal B.P. and Heart Rate (HR)

Accusure TD-3127 system, Taiwan was used for BP recording and an average of three reading was taken.

Heart Rate Variability (HRV)

Lead II ECG recording was done at lying posture, at a speed of 25 mm/sec and voltage of 10 mm/MV for 330 seconds to obtain HRV using data acquisition system RMS- Polyrite D version 2.2.

Power spectral analysis of data was done using fast fourier transformation (FFT). For recording of short term HRV, recommendation of Task force on HRV was followed [7].

Both the frequency domain and time domain analysis of the data was done.

Frequency domain analysis was performed using non parametric method of FFT. HRV software used a peak algorithm to find R wave which was done at a resampling rate of 4 Hz, minimum 256 data was required to perform spectral analysis to obtain 256 data a duration of 5 min ECG recording was done.

By frequency domain analysis following parameters were obtained, LF and HF power percentage, LF and HF power in normalized units (nu) and LF/HF ratio.

From the same data time domain measures of HRV were also obtained and the following parameters were calculated :

i. SDNN – Standard deviation of NN interval.

ii. RMSSD – Mean standard deviation – It is the square root of the mean squared differences of successive mean NN intervals deviation from arithmetic mean.

iii. NN50 – NN50 is the number of consecutive RR interval differing more than 50 ms.

iv. pNN 50 – It is the percentage of NN 50 intervals.

v. Variance – Variance is a measure of Statistical dispersion.

Hand grip test (HGT)

For isometric exercise test a light and small handgrip dynamometer was used

Before the test baseline BP was recorded in sitting postures then the subject were instructed to press the dynamometer with their dominant hand with maximum possible force. This gave the value of maximum voluntary contraction (MVC). The 30% of the MVC was calculated and then the subjects were instructed to press the dynamometer continuously at 30% of their MVC for 4 minutes by their dominant hand. BP was recorded at 1^{st}, 2^{nd} and 3^{rd} minute of isometric contraction and the 6^{th} minute value was measured as recovery after termination of isometric contraction. The systolic and diastolic BP differences from resting value were calculated at 1^{st}, 2^{nd} and 3^{rd} minute.

Cold pressor test (CPT)

Prior to the test baseline BP was recorded. The subjects were asked to immerse their hand up to the wrist in 4.5 C cold water for 2 min. BP was recorded from the other arm at 1^{st} and 2^{nd} min. of immersion and 5^{th} minute value was recorded as recovery.

The change in diastolic and systolic pressure was calculated at 1^{st} and 2^{nd} minutes from the resting value.

Lying to standing test (LST)

The subjects were asked to stand from supine position in 2 to 3 seconds then stood steady for 2 min. Blood pressure measurement was done at 0.5^{th}, 1^{st} and 2 min. The changes in systolic and diastolic pressures were calculated at 0.5^{th}, 1^{st} and 2^{nd} min of measurement after standing up.

Valsalva maneuver (Valsalva ratio)

Valsalva maneuver was performed on a mercury manometer which was locally assembled in our laboratory. In this test subject were asked to raise the intra thoracic pressure to 40 mmHg through a mouthpiece connected to mercury manometer and maintain at this level for 15 sec with nose clip on their nose.

Continuous EKG recording was done on BPL electrocardiograph Cardiart 108T-DIGI for 1 min before straining, for 15 seconds during straining and 45 seconds after the release of strain. Valsalva ratio is calculated by taking longest R-R interval during phase IV to the shortest R-R interval during phase II.

Deep breathing test (E: I ratio)

The subjects were give continuous signal in the form of raising the hand continuously up and then bringing the hand continuously down corresponding to inspiration and expiration to their full capacity without breaking the breath during inhalation and exhalation. The frequency of the cycle was 6 breaths/min for 1 minute. Phases of respiration were matched manually on ECG. The average of six widest R-R intervals was measured during expiratory phase and similarly six shortest R-R intervals were measured during inspiratory phase. The averaged value of expiratory R-R interval and inspiratory R-R interval was taken to calculate the E: I ratio.

Aerobic exercise

Subjects performed aerobic exercise on treadmill following a modified bruce protocol (8). Subjects performed treadmill exercise according to the modified Bruce protocol till the fourth stage (8).

Antioxidant status

Antioxidant status was assessed by measuring the level of malondialdehyde in plasma which was done by spectrophotometeric method. MDA was quantified by Cell Biolabs' TBARS Assay Kit (9). MDA forms a 1:2 adduct with thiobarbituric acid. The MDA-TBA adduct formed from the reaction of MDA in samples with TBA was measured. The unknown MDA containing samples or MDA standards were first reacted with TBA at 95°C. After a brief incubation, the samples and standards were read spectrophotometrically. The MDA content in unknown samples was determined by comparison with the predetermined MDA standard curve. The values were reported in nmol.

Statistical analysis of the data

The comparison for the subjects was done between the pre and post training within the group by using paired t test or non parameteric Wilcoxon matched-pairs signed-ranks test depending on the distribution of the data. Pearson's correlation was applied to see the correlation between autonomic function and antioxidant status. Statistical analysis was done using Graph Pad Instat 3.10, 32 bit (Graph Pad software Inc.) for windows.

Results

Table I shows that the mean SBP and DBP were 117.30±3.13 mmHg and 74.42±4.66 mmHg respectively before the exercise. After three weeks of exercise, mean SBP and DBP were reduced to 115.53±2.81 mmHg and 73.34±3.79 respectively. When the post exercise value of SBP and DBP was compared with pre exercise value, this reduction was statistically significant. (p=0.0201 for SBP) (p=0.045 for DBP).

Table II shows that LF nu variable showed a highly significant decrease (p=0.0014) from pre exercise (84.52±16.09) to post exercise value (70.48±20.89).

While HFnu variable showed a highly significant increase (p=0.0064) from pre exercise (14.905±5.44) to post exercise value (18.57±6.46).

A decrease in LF power percentage and LF: HF ratio

TABLE I: Effect of aerobic exercise on sympathetic activity.

Variables	*Group (I)*		*P value*
	Pre-exercise Mean±SD	*Post-exercise Mean±SD*	
SBP (mmHg)	117.30±3.13	115.53±2.81	0.0201
DBP (mmHg)	74.42±4.66	73.34±3.79	0.045

SBP-systolic blood pressure, DBP-diastolic blood pressure.

TABLE II: Effect of aerobic exercise on frequency Domain measures of HRV.

Variables	*Group (I)*		*P value*
	Pre-exercise Mean±SD	*Post-exercise Mean±SD*	
LF (%)	50.44±12.02	46.42±12.62	>0.05
HF (%)	22.17±8.82	24.40±9.45	>0.05
LF: HF ratio (%)	2.69±1.19	2.26±1.28	>0.05
LF nu	84.52±16.09	70.48±20.89	0.0014
HF nu	14.90±5.44	18.57±6.46	0.0064

LF-Low frequency power percentage, HF- High frequency power percentage, LF/HF- Low frequency and High frequency ratio. LFnu and HF nu-Low frequency and High frequency in normalized units respectively.

and increase in HF power percentage showed a trend towards increase in parasympathetic activity after aerobic exercise.

Table III represents blood pressure response to :

Hand grip test (HGT). The delta value of SBP and DBP were 20.65±6.57 mmHg, 21.82±6.70 mmHg respectively before the exercise. After three weeks of exercise delta value of SBP and DBP were 19.14±4.56 mmHg and 19.63±5.93 mmHg respectively. When the post exercise delta value of SBP and DBP was compared with pre exercise value, this reduction was statistically not significant (p>0.05).

Cold Pressor test (CPT). The delta value of diastolic blood pressure response to cold pressor test reduced from pre exercise value of 20.24±5.38 mmHg to 17.76±5.28 mmHg after aerobic exercise. This reduction was statistically significant (p=0.01198).

The reduction in delta value of systolic blood pressure response during cold pressor test before the exercise (20.44±4.61 mmHg) and after aerobic exercise (18.36±5.32 mmHg) was insignificant.

Lying to standing test (LST). The delta value of SBP and DBP were 8.73±3.57 mmHg and 10±4.25 mmHg respectively before the exercise. After three weeks of exercise delta value of SBP and DBP were 8±4.10 mmHg and 8.93±4.61 mmHg respectively. When the post exercise delta value of SBP and DBP was compared with pre exercise value, this reduction was statistically not significant (p>0.05).

TABLE III : Effect of aerobic exercise on sympathetic reactivity.

Variables		*Group (I)*		*P value*
		Pre-training Mean±SD mmHg	*Post-training Mean±SD mmHg*	
HGT	SBP	20.65±6.57	19.14±4.56	>0.05
	DBP	21.82±6.70	19.63±5.93	>0.05
CPT	SBP	20.44±4.61	18.36±5.32	>0.05
	DBP	20.24±5.38	17.76±5.28	0.0119
LST	SBP	8.73±3.57	8±4.10	>0.05
	DBP	10±4.25	8.93±4.61	>0.05

HGT - Hand grip test, CPT - Cold pressor test and LST - Lying to standing test. The data represents the delta values for systolic blood pressure (SBP) and diastolic blood pressure (DBP) for the reactivity tests performed.

Table IV shows the levels of Malondialdehyde (MDA) in the plasma 3.051±1.488 nmol before the exercise. After three weeks of exercise, the MDA levels were reduced to 2.422±1.102 nmol. When the pre exercise value was compared with post exercise value, this reduction was statistically significant (p=0.0179).

Table V shows that when the value of MDA was correlated with sympathetic and parasympathetic parameters no significant correlation was observed.

TABLE IV : Effect of aerobic exercise on antioxidant status.

Test	*Group (I)*		*P value*
	Pre-exercise Mean±SD	*Post-exercise Mean±SD*	
MDA (nmol)	3.05±1.48	2.42±1.10	0.0179

TABLE V : Correlation of MDA with activity parameters.

Variables	*r value*	*p value*
SBP (mmHg)	0.14	0.55
DBP (mmHg)	0.33	0.15
SDNN (ms)	–0.19	0.40
RMSSD (ms)	–0.35	0.12
NN50 (count)	0.38	0.08
PNN 50 (%)	0.09	0.67
CV (ms^2)	–0.36	0.11
LF (%)	0.28	0.22
HF (%)	–0.02	0.92
LF: HF ratio (%)	–0.04	0.84
LF nu	0.16	0.48
HF nu	–0.19	0.41

Discussion

The present study was planned with an aim to find out how aerobic exercise helps in maintaining physical fitness in terms of autonomic functions and to find out whether they have any impact on antioxidant status. Autonomic functions have been very well documented in the literature to improve both in subjects/patients performing routine aerobic exercise. It has been shown previously as well that even a moderate exercise training for few weeks results in favorable autonomic responses (10). We also undertook this study to see the autonomic responses and antioxidant status after a short duration of moderate physical exercise training on young healthy adults.

In our study autonomic activity as measured by SBP & DBP showed a significant decreased after the physical training exercise. We observed a favorable and statistically significant change in the frequency domain analysis of HRV. LF decreased and HF increased significantly after 3 weeks of aerobic exercise training, showing an increase in parasympathetic tone and a decrease in sympathetic tone. A significant decrease in delta value DBP was observed in CPT. We also observed a significant increase in antioxidant status after aerobic exercise training.

Our results are in concordes with the study performed by Sormers VK et al (11), which shows that blood pressure falls after short duration of exercise training in normo and hypertensive's.

Diastolic blood pressure is a direct measure of sympathetic influences on the vasculature but the vasculature itself is under multiple modulating influences apart from SNS during exercise. It has also been observed in our lab that initial reductions in sympathetic reactivity could be precursor for later decline in resting diastolic blood pressure. This indicates gradual reduction in sympathetic outflow to the vasculature.

The present study did not reveal significant effect on time domain measures of HRV but the trend towards increase in time domain measures was observed.

There were significant changes in the frequency domain measures of HRV. Increase in parasympathetic nervous system activity was observed by significant increase and decrease in HFnu and LFnu respectively there by decrease the LF/HF ratio significantly.

Heart rate variability (HRV) is now considered as an important tool to assess the influence of resting autonomic tone on cardiovascular system. It is now known that among the prominent frequency bands in HRV frequency spectra, high frequency (HF) component is attributed to parasympathetic influences on the heart and low frequency (LF) component is due to both PNS and SNS activity. There was a trend towards an increase of PNS activity as measured by LF: HF ratio, absolute HF and HFnu. This trend may be a forerunner of increased PNS activity (tone) for a longer duration of physical training. Time domain measures of HRV showed an increase in mean RR interval, SDNN, RMSSD, CV, NN50 and pNN50 similar to Sharma et al (10).

Hautala et al 2003 (6) showed that there is a relationship between HF and training response suggesting that cardiovascular autonomic regulation is an important determinant of training response to physical exercise.

Goldberger et al 2006 (12) validated RMS (equivalent to SD of NN intervals after linear detrending of the NN intervals) and MSSD parameter, reflect parasympathetic reactivation during post exercise recovery period. Jason et al (13) also validated this point and also proved the â adrenergic effect.

The cold pressor test (CPT) showed a significant decrease in diastolic blood pressure response after training (delta value) while delta value of SBP was also lowered.

Hand grip test (HGT) and lying to standing test also showed a decreased in delta value of SBP and DBP.

Both the handgrip test (HGT) and cold pressor test (CPT) showed lowered diastolic blood pressure responses after physical training (10).

Winder et al (14) showed a decrease in catecholamines responses to heart rate in acute exercise in men after 3 weeks of physical training. In certain diseases, short-term exercise training is known to affect cardiorespiratory parameters and aerobic capacity.

Sormers VK 1991 (11) has shown that both in normotensive and hypertensive populations, exercise training results in reduction of resting diastolic blood pressure at different duration of physical training. Central command arising from the mesecephalic locomotor region (MLR) as well as the exercise pressor reflex is capable of resetting the carotid baroreflex response during exercise. Resetting probably occurs rapidly at the start of exercise and most likely is initiated by central command. Once the muscles have started to contract, the exercise pressor reflex contributes to resetting as well (15). It has now been well documented that exercise pressor reflex is one of the important contributor in changes in blood pressure response seen after regular physical training. Autonomic nervous system is one of the most important mediator of this response and these changes may be responsible for the present observations in HGT and CPT.

Therefore reduced sympathetic reactivity response to CPT and HGT is due to training effect. Repeated rise of blood pressure during exercise training may have activated negative feedback mechanisms resulting in lowered diastolic blood pressure responses to these tests.

There was no statistically significant change in terms of parasympathetic reactivity parameters like E: I ratio and Valsalva ratio.

It has been documented that parasympathetic reactivity changes are slow to develop than sympathetic reactivity (10). Our 3 weeks exercise period was not enough to cause a significant change in the parasympathetic reactivity.

A significant change was observed in the plasma level of malondialdehyde (MDA) after a period of three weeks of aerobic exercise. Robertson et al. (1991) (16) had also observed a high antioxidant status in trained runners than sedentary individuals. Toskulkao et al. (1996) (17) observed a higher erythrocyte enzyme activity in trained runners than untrained subjects. Priscilla M Clarkson et al. (2000) (18) had observed that exercise training seems to reduce the oxidative stress of exercise, such that trained athletes show less evidence of lipid peroxidation for a given bout of exercise and enhanced defense system in relation to untrained subjects.

Conclusion

Our results are similar with the findings in the literature and it reinforces the fact that even a short duration of physical exercise of three weeks can result in a favorable change in the biochemical parameters such as MDA. We had initiated this study to validate a very important physiological effect of a moderate exercise training on the autonomic functions as well as on the antioxidant status, though the literature tells us about the various observations of the of moderate exercise training on the autonomic functions, but there are very few studies which have taken into account the antioxidant status along with autonomic functions. Our results support the earlier observations that even moderate physical exercise training can result in favorable physiological and biochemical outcome. Indulgence in regular physical exercise can result in betterment of health in general and improvement in autonomic functions and antioxidant status in particular.

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