



The Effect of Different Exercise Intensities on Plasma Endostatin in Healthy Volunteers

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Authors' contributions

This work was carried out in collaboration among all authors. Author IS designed the study, wrote the protocol and performed statistical analysis. Author TA wrote the manuscript. Author RHB helped in designing the idea and data analysis, critical review. Authors MI and AK managed literature search and helped in manuscript corrections. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i1331263

Editor(s):

(1) Dr. R. Deveswaran, M. S. Ramaiah University of Applied Sciences, India.

Reviewers:

(1) Kijin Kim, Keimyung University, Korea.

(2) Maciste H. Macias, University of Guanajuato, México.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/65322>

Original Research Article

Received 10 December 2020

Accepted 17 February 2021

Published 18 March 2021

ABSTRACT

Background: Physical activity decreases the risk and development of many angiogenesis related health problems including atherosclerosis. Physiological influence of different physical activities on plasma endostatin concentration is contradictory. This study aimed to determine the effects of mild, moderate and vigorous exercise on the concentration of endostatin.

Methodology: 22 participants, 16 males (age = 30.6 ± 7.8 years) and 6 females (age = 26.5 ± 5 years) were recruited. Weekly session of different intensities exercise based on predicted maximum heart (60% (low), 70% (moderate) and 80% (vigorous)] were carried out. One pre and two post exercise samples were taken at 10 and 60 minutes.

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Results: Low intensity exercise insignificantly decreased the endostatin concentration at 10 and 60 minutes ($P = 0.5$ and 0.8). However, moderate (P values = 0.022 , 0.004) and vigorous intensities ($P = < 0.001$, 0.02) increased the endostatin concentrations significantly at both intervals respectively. The effects were not significantly influenced by gender, exercise mode (walking vs running), components of exercise (HR, Speed, Gradients, distance, duration) or metabolism during exercise (VO_2 max, VCO_2 , RER, Energy expenditure).

Conclusion: Low intensity exercises did not influence endostatin concentration. However, moderate to high intensity exercises significantly increase endostatin concentration and may have potential benefits.

Keywords: Exercise; angiogenesis; endostatin.

1. INTRODUCTION

The importance of physical activity for decreasing the risk of many chronic diseases including cardiovascular disease and diabetes is an established fact. Enhanced physical activity do so through different mechanisms including local cardiovascular adaptations such as production of heat shock proteins and increasing anti oxidant capacity [1] as well as modifying other risk factors such as obesity and diabetes through increasing insulin sensitivity and changing the lipid profile [2,3]. These are achieved through many mechanism including blood related, hormonal, neuronal and local adaptations [1]. Off the local adaptations, extension of collateral circulation is one of the important determinants. Controlled angiogenesis expands the vascular network by formation of new vessels [4]. However, uncontrolled angiogenesis is dangerous in increases the size of atherosclerotic plaque and can make it vulnerable to rupture [5]. During such conditions, the roles of angiostatic mediators become important, as they tend to halt the process.

Exercise affects the angiogenesis by increasing or decreasing the circulating levels of angiogenic mediators. The over expression of circulating VEGF (key angiogenic mediator) in myocardium [6], skeletal muscles [7] and plasma [8] and endostatin (Key angiostatic mediator) after exercise has been reported.

Exercise has also been reported to influence Endostatin (potent inhibitors of angiogenesis) differently both by increasing [9,10] and decreasing its concentration [11]. Although the exact mechanism, by which exercise influence endostatin is not completely, understood, the differences in exercise protocols, intensities, durations and physical status of the participants could be considered for these variations in results. In order to have a better understanding

of how exercise influences the circulatory endostatin concentrations, an exercise protocol to use 3 different intensities at 60, 70 and 80% of the predicted maximum heart rate with constant energy consumption (200 kcal) for each session was proposed.

2. MATERIALS AND METHODS

A total of 22 volunteers (16 male and 6 female) in the age group 18-45 years, non smokers with no relevant cardiac history, not on medication, not pregnant or lactating and asymptomatic otherwise were recruited after screening for exercise fitness through the Physical Activity Readiness Questionnaire (PAR-Q). Their characteristics are given in Table 1.

2.1 Protocol of the Study

The participants visited the lab on 4 occasions, 7 days apart. On visit one the participants underwent for measurement of a 20 minutes sub maximal exercise test using a walking or running protocol based on individual fitness levels. At the start of the test, all volunteers stood on the treadmill at rest for 5 minutes. After this, the first stage of the test was started based on selecting a comfortable speed (4 to 5 km/hr for walking group and 6 km/hr for running group) for each participant. If it was a walking protocol, the gradient of the treadmill was increased in each subsequent stage by 3% after every 5 minutes. In case of a running protocol, speed was increased by 1 Km/hr. During each test, expired air was collected using Douglas bags and analyzed for substrate metabolism and energy expenditure, while speed during exercise was plotted against the heart rate at 4 different levels and extrapolated to predicted maximum heart rate from where required speed at 60%, 70% and 80% of their predicted maximum heart rate was calculated. In order to standardize the effort during each exercise session, energy

expenditure for each session was kept constant at 200Kcal per session. Therefore duration of each session varied as per individual's energy expenditure rate. Briefly duration of the exercise had inverse correlation with the speed of the participants. among all participant 9 participants including all females adopted the walking protocol based on their fitness and comfort level.

On Visit 2, 3 and 4 the participants performed exercise at 60%, 70% or 80% of their predicted maximum heart rate. Blood samples for endostatin were taken before and 10 and 60 minutes after each exercise session.

The order of visits 2, 3 and 4 were randomized for standardization. Blood was centrifuged within 30 minutes of collection and was later on analyzed through Quantikin® ELISA human endostatin kits (R&D systems for Europe, Abingdon, UK). It is a quantitative sandwich ELISA kit, which works on the principal of two antigenic determinants directed against two monoclonal antibodies.

2.2 Statistical Analyses of the Data

Statistical analyses were carried out using SPSS 17 and MINITAB 16 statistical software. Independent and sample Paired sample T-tests and One-way ANOVA with Bonferroni post hoc analyses with repeated measures were applied to determine the mean differences in groups and changes over time, before and after exercise.

Correlations of different variables with plasma endostatin concentration were done using Pearson correlation tests. Simple linear regressions were carried out for uni-variate and multivariate analyses to check any association of change in mean endostatin concentration with different exercise indices.

3. RESULTS

All the participants completed the study and no drop out were observed. The anthropometric data revealed that men were significantly taller ($P < 0.001$) and heavier ($P < 0.001$). as given in Table 1.

3.1 Endostatin Concentration in Plasma before and after Exercise at Protocol 1

During this stage participant performed exercise at 60% of PMHR. The mean speed during this session for all participants were 5.01 ± 0.1 km/hr

with a significant male to female difference (5.3 ± 0.7 vs. 4.2 ± 0.3 at 0° inclination, $P = 0.002$). The mean duration of exercise for all participants were 42 ± 15 minutes with significant male to female difference (35 ± 9 vs. 61 ± 11 , $P = < 0.001$).

Exercise at this stage decreased the mean endostatin concentration (101 ± 20 ng/ml Vs. 97 ± 22 ng/ml and 98 ± 23 ng/ml), 10 and 60 minutes after corresponding . These are equal to 4% and 3% ($P = 0.5$ & 0.8), as shown in Fig. 1. The difference was not influenced by gender or mode of exercise (walking vs running).

3.2 Endostatin Concentrations in Plasma before and after Exercise at Protocol 2

During this stage participant performed exercise at 70% of PMHR. The mean speed during this session for all participants were 5.8 ± 1.2 km/hr with a significant male to female difference (6.3 ± 1 vs. 4.2 ± 0.3 at 3.7° inclination, $P = < 0.001$). The mean duration of exercise for all participants were 30 ± 11 minutes with significant male to female difference (24 ± 5 vs. 45 ± 8 , $P = < 0.001$).

It was found that 77% of the individuals exhibited an increase in endostatin concentration while 23% exhibited a negative change at 10 minutes after exercise. However, at 60 minutes interval, the concentration raises for all participants.

Mean endostatin concentration in plasma at rest was 88 ± 22 ng/ml. Mean endostatin concentration was 105 ± 30 ng/ml at 10 minutes interval and 106 ± 33 ng/ml at 60 minutes interval. One-way ANOVA with repeated measures showed a significant difference in the mean endostatin concentration at rest and subsequent time points ($P = 0.002$). Bonferroni post hoc analyses revealed significant rise in plasma endostatin concentration at both time points after exercise ($P = 0.02$ & 0.004). This increase was 18% and 19% respectively, as shown in Fig. 2.

3.3 Endostatin Concentration in Running and Walking Group

The exercise increased plasma endostatin concentration significantly in both groups ($P = 0.014$ & 0.017) but the increase in the running group was more pronounced. In the running group an increase of 28% ($P = 0.05$) at 10 minutes interval and 26% ($P = 0.04$) at 60

minutes interval was observed. However, in the walking group, the rise in endostatin concentration was 9% ($P = 0.31$) at 10 minutes interval and 14% ($P = 0.02$) after exercise.

3.4 Exercises at 80% Heart Rate (Protocol 3)

3.4.1 Endostatin concentration in Plasma before and after exercise at protocol 3

During this stage participant performed exercise at 70% of PMHR. The mean speed during this session for all participants were 6.4 ± 1.9 km/hr with a significant male to female mean difference (7.2 ± 1.6 vs. 4.2 ± 0.3 at 7.2° inclination, $P < 0.001$). The mean duration of exercise for all participants were 23 ± 8 minutes with significant male to female difference (19 ± 4 vs. 34 ± 7 , $P < 0.002$).

The mean endostatin concentration at rest was 100 ± 20 ng/ml. One-way ANOVA with repeated measures showed a significant increase in mean endostatin concentration after exercise ($P < 0.001$). The mean endostatin concentrations at 10 and 60 minutes intervals were 138 ± 34 ng/ml and 114 ± 34 ng/ml respectively. These increase in means are equal to 38% ($P < 0.001$) and 14% ($P = 0.021$). Box plots of the pooled data are shown in Fig. 3. The difference in endostatin concentration was not influenced by gender or exercise mood as given in Fig. 3.

3.5 Correlations of Changes in Endostatin Concentrations and the Different Parameters of the Exercise at All Intensities

The difference in the endostatin concentration (Δ ES1) at baseline and 10 minutes after exercise, and (Δ ES2) baseline and 60 minutes were calculated for all the 3 protocols and correlated with different parameters of the exercise. No significant pattern was observed.

4. DISCUSSION AND IMPLICATIONS

This study was designed to investigate the impact of structured exercise activities on the endostatin concentration which is one of the key regulators of angiogenesis in general and formation of collaterals in case of ischemic events. In more specific terms, this study examined the impact of 3 different exercise intensities (mild, moderate and vigorous) on the

concentration of endostatin in plasma of young healthy adult male and females. It was aimed that different exercise intensities might influence the endostatin concentration in plasma differently. Moreover, it was also aimed, that gender and variations in physical characteristic might be differently influenced.

4.1 Exercise at Protocol 1

This study found that endostatin concentration was negatively altered with low intensity exercise. During this exercise the participants lightly walked for relatively longer distance over reasonably longer durations. Though exercise minimally decreases the mean endostatin concentration but 64% of the individuals showed a transient decrease after the intervention. The decrease in endostatin concentration acutely after exercise has not been reported previously.

However a significant decrease in the basal endostatin concentration after 06 months of endurance training for 3 episodes of 90 minutes walking or 60 minutes cycling in overweight men [11]. Nonetheless, this study fails to report the time duration between the last exercise session and the blood samples taken for endostatin analyses. Similarly, no difference was reported in endostatin concentrations for healthy adults after high mountain climbing [12]. Another Study reported a non significant and minimal increase in the plasma endostatin concentration after 30 minutes of walking on treadmill at 55-59% maximum heart rate intensity [13]. The decrease in endostatin concentration in the skeletal muscles of the mice, after 04 weeks of moderate exercise for 30 minutes a day for 7 days a week was reported [14].

The exact mechanism of why endostatin concentration decreases after low intensity exercise is, however, poorly defined. Endostatin is inversely correlated with the capillary density in the skeletal muscles and that exercise tends to improve the capillary density, in turn decreases the endostatin concentration in the skeletal muscles [14]. Whereas, [11] postulated that long term exercise decrease the circulating endostatin with two possible effects; firstly with the decrease in endostatin concentration a more angiogenic phenotype in body muscles is enhanced and secondly in case of high intensity physical activity, a rapid increase in the circulatory endostatin provides the beneficial effects against atherosclerosis. However, no evidence is there to back this hypothesis and further experimental work would be required to prove this.

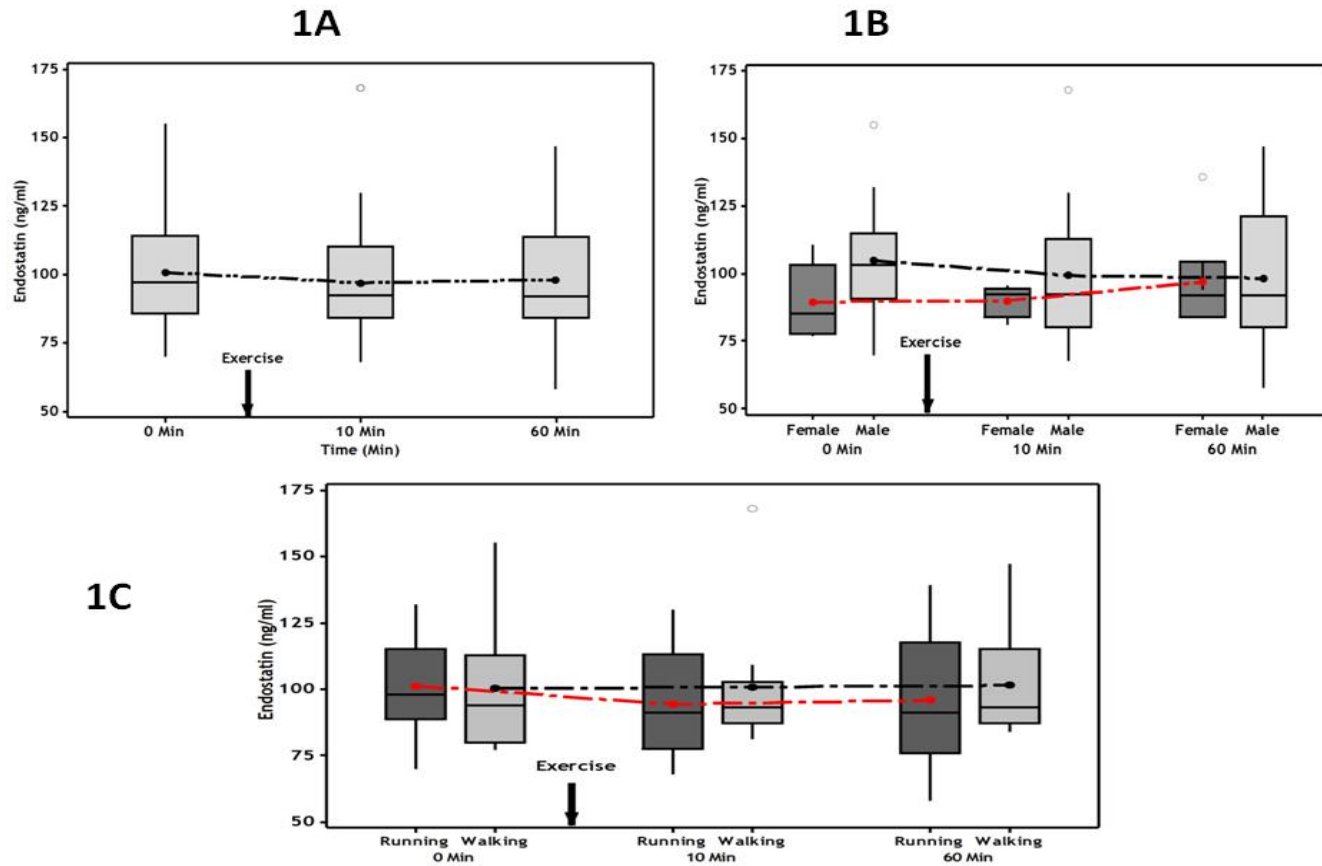


Fig. 1. Endostatin concentrations before and different intervals after exercise at protocol 1 for all data (1A), on the basis of gender (1B) and mode of exercise (1C)

Box plots illustrating the mean, median, quartile range and range of endostatin concentration in plasma before and 10 and 60 minutes after exercise at protocol 1. The clear circle represents outlier. ANOVA with repeated measures show no significant changes in mean endostatin concentrations at any time point ($P = 0.5$)

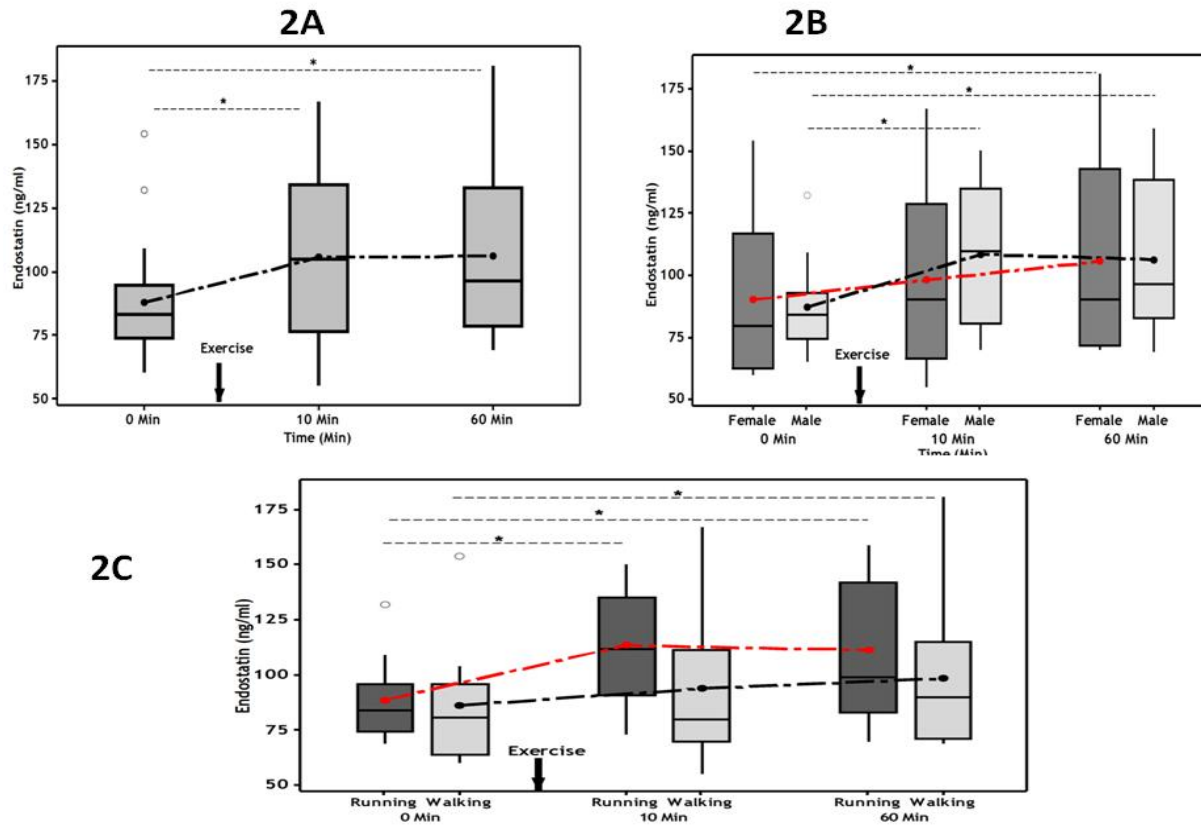


Fig. 2. Endostatin concentration before and different intervals after exercise at protocol 2 for all data (2A), on the basis of gender (2B) and mode of exercise (1C)

Box plots illustrating the mean, median, quartile range and range of endostatin for all participants before and 10 and 60 minutes after exercise at protocol 2. Two participants presented with high endostatin concentrations in plasma at rest and are shown as outlier by clear circles. The mean endostatin concentrations increase by 18% ($P = 0.02$) and 19% ($P = 0.004$) respectively, at 10 and 60 minutes after exercise and shown by the dotted line with*

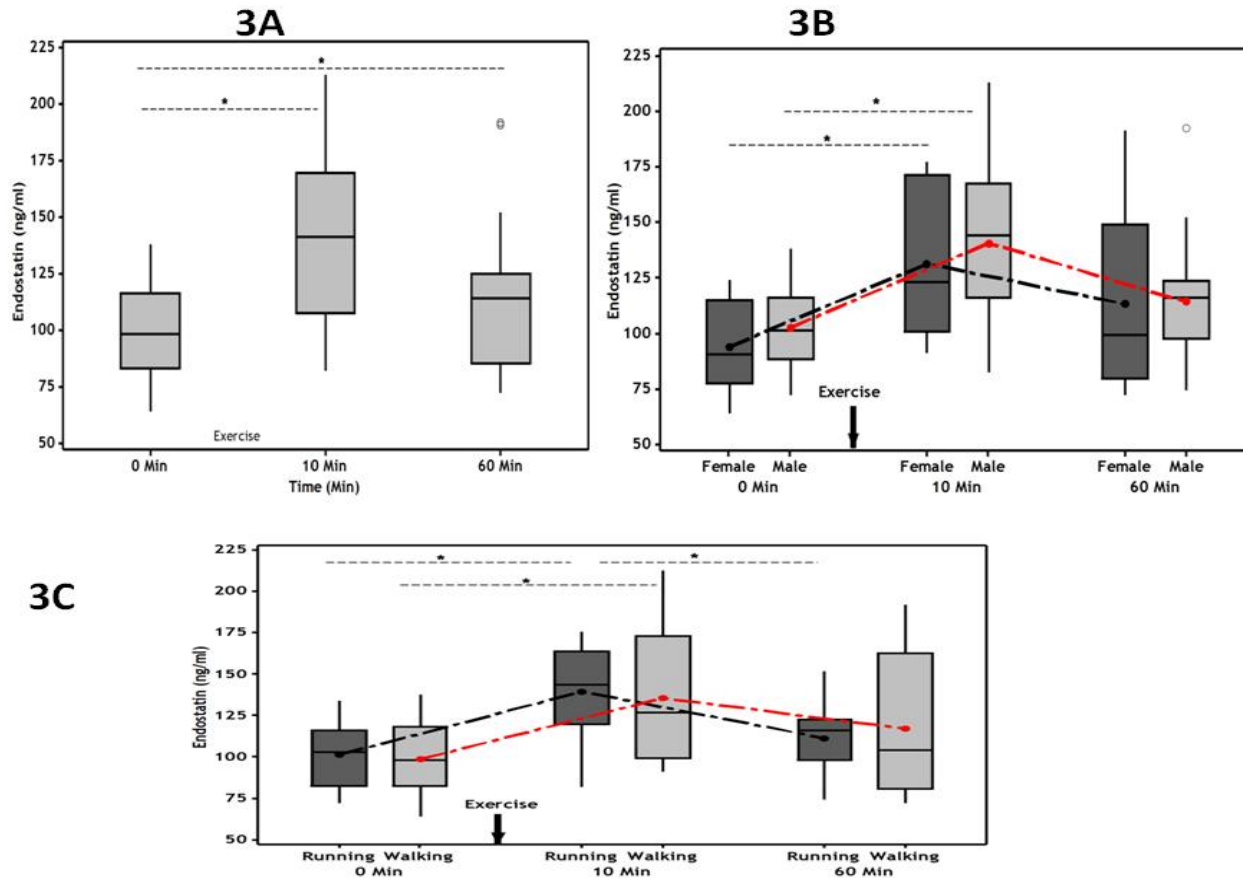


Fig. 3. Endostatin concentration before and different intervals after exercise at protocol 3 for all (3A), On the basis of gender (3B) and mode of exercise (3C)

Box plots illustrating median, quartile range and range of endostatin concentration in plasma before and 10 and 60 minutes after exercise at protocol 3. Significant changes in endostatin concentrations were observed at 10 minutes ($P < 0.001$) and 60 minutes ($P = 0.021$). Clear circles represent outlier

4.2 Exercise at Protocol 2 and Protocol 3

A steady and significant increase in endostatin concentration in plasma was observed after the A steady and significant increase in endostatin concentration in plasma was observed after the exercise at protocol 2. The increase in the mean endostatin was 18% and 19%, 10 minutes and 60 minutes after the exercise respectively ($P = 0.02$ & 0.001). There was no sex specific difference in increase, although the pattern of increase between males and females was slightly different. Male showed an initial significant increase of 24% followed by a slight decrease to 22% at 60 minutes interval while females showed a steep increase of 9% initially which increased to 17% at 60 minutes. Contrasting to the results in previous section, 77% of the participants exhibited a positive change in endostatin concentrations after exercise while 23% showed a negative change, as illustrated in figure 03. However, it can be seen that after initial decrease, all participants exhibited positive changes.

Similar results were obtained when the volunteers performed the 3rd physical activity at higher intensity (protocol 3). The increase in mean endostatin was 38% at 10 minutes interval which remained higher even after 60 minutes of the exercise. There was no sex specific difference in mean concentrations. Concurrently, no differences between the running and walking groups were observed.

The results in this section of the study confirm and expand the earlier observations of increase in plasma endostatin concentration after a moderate to high intensity exercise by different research groups [9], [15,10] and [8]. Gu and his colleagues reported increase up to 73% increase for 6 hrs post exercise. The difference could possibly be due to the small sample size by Gu ($n = 7$) and only male participants in the study. If male to male comparison is carried out, the highest increase for males in our study was 38% at 10 minutes interval.

Results in this study are different from the study done by [11], who reported a decrease in plasma endostatin concentration after 6 months of exercise training and [13], who did not find any increase in endostatin concentration after exercise at 55-59% heart rate in females. The difference in design, intensity and timings of the samples taken could possible explain the difference in results.

Just as the walking and running groups, analyses were also carried out on the bases of BMI. Participants were divided into normal ($BMI < 25$) and overweight ($BMI > 25$) groups (data not shown). No differences in endostatin concentration of plasma were found between the two groups before or after the exercise at any time point during all the trials. Only one previous study has compared the increase in endostatin concentration for normal and obese females without any difference between them [13].

Moreover, as the change in endostatin with high intensities of exercise was significant, correlations of the different components with the change at different intervals after exercise were carried out. No prominent pattern of correlation was observed. Though, respiratory exchange ratio and rate of fat oxidation showed significant negative correlation with change in endostatin concentration at low intensity exercise. But these could not be reproduced for other exercise intensities. Gu find a strong positive significant correlation ($R^2=0.94$) between peak oxygen consumption and % change in endostatin consumption [9].

Based on the results, we can say that a threshold mechanism required for the release of endostatin after exercise, does exist and low intensity does not affect that threshold. Person to person variation in this threshold mechanism might explain the difference in influence by the same intensity of exercise. Moreover, it is also possible that decrease in venous endostatin concentration after exercise may be due to uptake of endostatin by tissue from circulation.

5. CONCLUSION

In conclusion, moderate and high intensity exercise acutely increases the endostatin concentration irrespective of gender or mode of exercise.

CONSENT

As per international standard or university standard, Participants' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

Approval for this experimental study was granted by the University of Glasgow, Ethics Committee.

ACKNOWLEDGEMENT

The first author acknowledges Khyber Medical University, Peshawar and Higher Education commission of Pakistan for providing financial support for PhD from University of Glasgow.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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