Histomorphometric adaptations of Superior and Inferior Venae Cavae after Moderate Intensity Continuous Exercise Training - A preclinical trial

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Abstract

Objective: This study was aimed at evaluation of effects of Moderate Intensity Continuous Training (MICT) on the histomorphometric parameters of superior vena cava (SVC) and inferior vena cava (IVC) in Sprague Dawley rats.

Methods: Twenty-four male healthy Sprague Dawley rats were randomly divided into two groups. Group A (n=12) was assigned to the Moderate Intensity Continuous Training whereas Group B (n=12) was the control group with no exercise. The exercise intervention was workout on treadmill at 70% Maximal exercise intensity for 22-24 mins for 5 consecutive days/week for four weeks. The animals were sacrificed after completion of exercise protocol for histomorphometry of the Inferior Vena Cava and Superior Vena Cava. H&E stained slides were examined under Nikon Ts2R-FL inverted microscope and images were taken. Images were analyzed and morphometry was performed by using Image J software. Luminal area, wall thickness and wall to lumen ratio were evaluated. Statistical analysis was performed on SPSS version 23.

Results: Histomorphometric evaluation of the luminal area, wall thickness and wall to lumen ratio of both large veins showed statistically significant difference between control and the exercise groups. The luminal area, wall thickness and wall to lumen ratio of the IVC was significantly increased in the exercise group with p values 0.003, 0.04 and <0.001 respectively. There was statistically significant increase in luminal area and wall thickness of SVC in the exercise group (p<0.0001) and (p = 0.0001), whereas wall to lumen ratio was found to be reduced significantly p<0.001.

Conclusion: Our study has revealed that moderate intensity continuous exercise of four weeks durati on has profound effects on the histomorphometry of superior and inferior vena cava. The findings of our study highlight that the venous morphological adaptations to meet the heightened demands gene rated after moderate intensity continuous exercise are as prevalent as cardiac and arterial adaptations.

Keywords: Exercise, Histomorphometry, Inferior Vena Cava, Superior Vena Cava, Moderate Intensity Continuous Exercise

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Introduction

Veins have an indispensable role in the overall well-being of an individual. The crux of their significance lies in the performing the elemental function of serving as conduit for circulating blood from body towards heart. In addition, veins serve to regulate blood pressure and temperature and maintain fluid balance[1]. Chronic venous insufficiency is caused by incompetence of the veins to efficiently carry blood resulting in pain, swelling, edema and appearance of tortuous veins on the surface of skin specially in the lower limbs[2]. Annual prevalence of chronic venous disorders has shown to be 2.6 % in females and 1.9% in males worldwide. Additionally, it is found to be more prevalent in females than males with 3 to 1 female predominance[3]. The commonly found venous insufficiency, varicose veins has shown to have varied geographical prevalence. Currently it has a global prevalence of 2-73% while in Pakistan it is affecting 16-20% of the population[4].

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It has been observed that diverse prevalent venous disorders respond very well to the conservative management incorporating exercise and physical activity of moderate intensity. Moderate intensity continuous exercise is the aerobic exercise consisting of medium intensity (64-76 % HR) exercise like jogging, cycling, or treadmill walking which lasts 20 min to an hour performed continuously without break[5]. Moderate Intensity Continuous Exercise has numerous effects on the structure and function of various components of the cardiovascular system. Regular Moderate intensity continuous training is found to reduce fibrosis in the left ventricle and promote vasculogenesis which is evident by increased capillary density[6]. Thus, evidence is available for exercise-induced histological changes in the arteries and induction of angiogenesis in the heart as well as the effects of exercise on cardiovascular system, in general. There are studies which indicate that exercise causes a reduced peripheral vascular resistance and increases venous return to the heart which is attributed to pumping action of the exercising muscles that propel blood towards the heart[7]. Thus, it can be stated, that exercise must be imparting its effects on the histological structure of all the components of the CVS (cardiovascular system) including large-sized veins. However, the effects inflicted by exercise on the histological structure of veins are not well understood. This scarcity of literature demands meticulous investigation on the said subject. Considering the histology with few variations three basic layers are found in the large veins i.e. tunica intima, tunica media, and adventitia. Tunica intima is the innermost followed by tunica media in the middle and tunica adventitia is the outermost layer. Moreover, in the assembly of each layer endothelial cells are the main component of the tunica intima, while smooth muscles forming the tunica media and tunica adventitia being thickest primarily consist of fibroblasts, collagen fibers, autonomic nerves, and vessels. In comparison to veins, arteries have thin tunica adventitia, thick tunica media with more organized smooth muscles, and narrow lumen. Veins in comparison to arteries have less elastin content in their walls which owes to lesser elasticity and the ability to hold relatively large volumes of blood at low pressure[8]. Despite this, Veins in contrast to arteries are regrettably rarely investigated particularly for their microstructure, which is noticeably different. Previously, superficial basilic vein and deep brachial were assessed with the help of ultrasound in the cyclists. Subjects performed cycling exercises at 60% of peak oxygen uptake. Results were very interesting as it was found that the cross-sectional area was increased in the superficial but decreased in the deep veins[9]. These results are suggestive of structural modifications of venous tissue after exercise. Similarly, it was explored through the ultrasonography that the diameter of the deep veins was found to be increased in trained athletes when compared with untrained population, suggesting the morphological alterations in the venous ultrastructure after exercise[10]. Authors of the same in their recently published study have raised concern over the scarcity of the literature that determine the effects of exercise on the venous morphology. However, most available evidence pertinent to veins is through hemodynamic studies. The aim of this study is to evaluate the histomorphometric alterations in parameters of superior vena cava and inferior vena cava after moderate intensity continuous exercise in rat model which can further be translated into humans.

Methodology

The investigation was carried out with approval obtained from the university's animal ethical committee (2022-04/FL/ZCRS). The study involved 24[11] healthy male Sprague Dawley Rats, aged young, and weighing between 200 and 300 grams. These rats were procured from the Animal House of the International Centre for Chemical and Biological Sciences. Sample size was calculated using the method provided by Arifin and Zahiruddin, 2017[12].

Through a process of simple random sampling, the rats were divided into two equal groups, labeled as Group A and Group B. Group A, comprising 12 rats, underwent moderate-intensity training at 70% of their maximal exercise intensity. This intensity was determined using a progressive graded test on a specifically designed treadmill, where the animals ran until they refused to continue. The maximal speed reached during this test was considered, and 70% of it was established as the moderate exercise intensity, following the protocol outlined by Ye et al. in 2019[11].

The exercise regimen for Group A consisted of 22-24 minutes of moderate-intensity continuous exercise for five consecutive days per week over a four-week period (as illustrated in Figure- 1). A five-minute warm-up and cool-down were incorporated at the beginning and end of each session [13]. On the other hand, Group B, the control group consisting of 12 rats, did not undergo any exercise. Both groups were housed individually in cages under controlled temperature and lighting conditions. The rats in both groups received the same standard stock feed, water, and bedding in the form of wood shavings.

[* File contains invalid data | In-line.JPG *]At the conclusion of the intervention, all rats were anesthetized and dissected to obtain samples of their inferior vena cava (IVC) and superior vena cava (SVC). These tissue samples were preserved in formaldehyde solution, followed by dehydration using increased concentration of isopropyl alcohol. Subsequently, the alcohol was removed from the tissue sections with a xylene solution, and tissue embedding was performed by leaving the sections in paraffin wax overnight. The resulting tissue blocks were then sectioned, yielding 5-micrometer-thick slices. Hematoxylin and Eosin (H&E) staining was applied to these sections. For microscopic analysis, slides were prepared and observed using a Nikon Ts2R-FL inverted microscope. Tissue analysis, encompassing vessel luminal area, wall thickness, and wall-to-lumen ratio, was conducted using Image J software. Three readings for each variable were taken, and the mean was calculated for analysis. All statistical analyses were performed using SPSS 23, expressing numerical variables as mean and standard deviation. The distribution of data was assessed with the Shapiro-Wilk Test. For normally distributed numerical variables, an independent sample t-test was employed, while the Mann-Whitney U-test was used for non-normally distributed data. A p-value <0.05 was considered significant for all statistical analysis, considering the 95% CI.

Results

After the preparation of slides, they were studied under microscope to assess the exercise intervention outcomes in experimental and control groups. Outcomes assessed were luminal area,

Fig 1. Exercise session on purpose-built motor driven treadmill

wall thickness and wall to lumen ratio of superior vena cava and inferior vena cava.

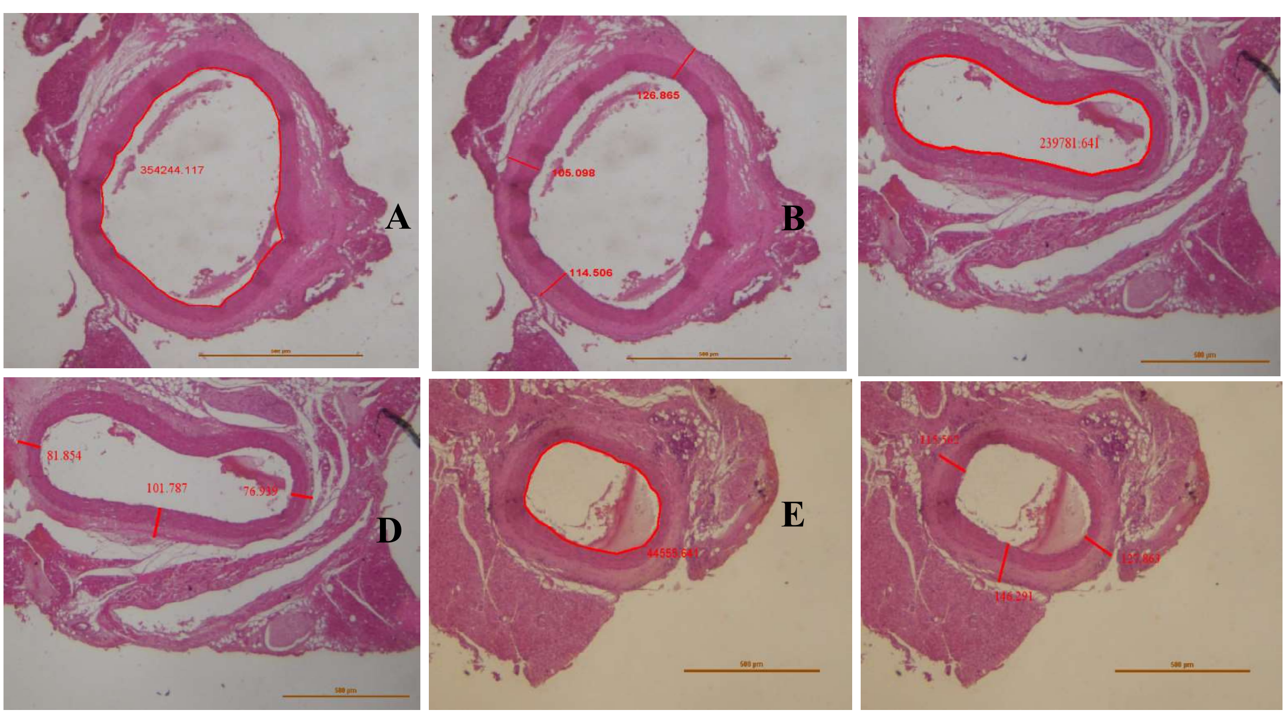
THe Luminal area of SVC in Exercise Group A (309571.11 μm^2 ± 34464.85) was significantly greater than luminal area Group B (136335.60 ± 77882.63). p-value ($p < 0.0001$) was suggestive of statistically significant difference using Mann-Whitney U Test. In inferior vena cava, the Luminal area of exercise Group A (191960.02 ± 44049.51) was also significantly greater than control Group B (110326.08 ± 49409.10). As p-value calculated by Mann-Whitney U Test ($p = 0.003$) was suggestive of statistically significant difference. Wall thickness (μm) of SVC exercise Group A (110.57 μm ± 13.36) was found to be significantly increased as compared to control Group B (82.39 μm ± 11.25). Using Mann Whitney U test p-value ($p= 0.0001$) was indicative of statistically significant difference between the groups. In IVC, Wall thickness of exercise group A (118.89 μm ± 7.22) was significantly increased as compared to Group B (104.62

μm ± 20.92). p-value (p = 0.04) calculated using independent sample t test was indicative of statistically significant difference between the groups. In SVC, Comparison of the means showed increase in the wall to lumen in the exercise group A (0.17 ± 0.01) when compared to control group B (0.12 ± 0.01). Hence, there was statistically significant difference between groups using independent sample t-test (p < 0.0001). In IVC, Comparison of the means of wall to lumen ratio showed decrease in the wall to lumen in the Group A (0.19 ± 0.03) when compared to Group B (0.28 ± 0.10). There was a statistically significant difference between groups (p < 0.001) by independent sample t-test. These results are illustrated in Table-1 and the photomicrographs of the superior and inferior vena cava are shown in figure – 2.

Table 1. Histomorphometric parameters of SVC and IVC in Exercise and Control groups

	Luminal Area (μm^2) † Mean± SD		**Wall thickness (μm) † Mean±SD**		**Wall to lumen ratio †† Mean±SD**	
Superior Vena Cava	**Exercise Group A**	**Control Group B**	**Exercise Group A**	**Control Group B**	**Exercise Group A**	**Control Group B**
	309571.113 ±34464.85	136335.60 ±77882.63	110.57 ±13.36	82.39 ±11.25	0.17 ±0.01	0.12 ±0.01
p value	p < 0.0001***		p= 0.0001***		p < 0.0001***	
	Luminal Area (μm^2) † Mean± SD		**Wall thickness (μm)†† Mean±SD**		**Wall to lumen ratio †† Mean±SD**	
Inferior Vena Cava	**Exercise Group A**	**Contro Group B**	**Exercise Group A**	**Control Group B**	**Exercise Group A**	**Control Group B**
	191960.02 ±44049.51	110326.08 ±49409.10	118.89 ±7.22	104.62 ±20.92	0.19 ±0.03	0.28 ±0.10
p value	p = 0.003**		p = 0.04*		p < 0.001***	

* p<0.05, **p<0.01, ***p<0.001†
Mann Whitney U test, †† Independent sample t test
SD = standard deviation



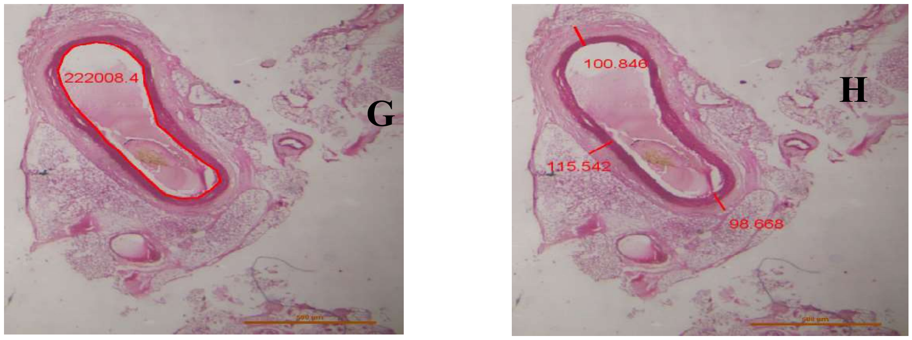


Fig 2. Pictomicrograph of H& E stained sections of Superior and inferior vena cava

A&B showing Luminal area and wall thickness of the Exercise group of superior vena cava of the rats. **C&D** showing Luminal area and wall thickness of the Control group of superior vena cava of the rats. **E&F** showing Luminal area and wall thickness of the Exercise group of inferior vena cava of the rats. **G&H** showing Luminal area and wall thickness of the Control group of inferior vena cava of the rats.

Discussion

Veins in comparison to arteries have not been extensively studied despite their indispensable role in overall cardiovascular function[20]. Therefore, to compare our results the pertinent data on the histomorphometric evaluation of veins particularly after exercise is extremely scarce. Results of our study showed a clear difference between the parameters of control and exercise both in superior and inferior vena cava. Luminal area of the vessels was found to be increased to a statistically significant level. In a study by Cheng and his team it was observed by using magnetic resonance imaging that wall shear stress, blood flow rate and cross-sectional area were greatly increased in inferior vena cava of the 11 human subjects after aerobic cyclic exercised[14]. This is in accordance with our findings where lumen area was found to be increased in the superior and inferior vena cava. Another venous parameter in our study was wall thickness, it was evaluated that wall thickness of both superior and inferior vena cava was remarkably increased in the exercise group as compared to the control group. Consistent with the findings of Salimi and co researchers where 50 females were evaluated by the ultrasonography after following a regimen of handgrip exercises. It was found that venous wall thickness along with the area was increased after following a plan of isometric hand grip exercises[15]. Although the results of the study are obtained by ultrasonography, they are clearly indicative of the histological adaptations in veins after exercise.

Relatable to our study was a research conducted through doppler ultrasound by Uy et al., 2012, it was expressed that there was an increase in the luminal diameter of cephalic vein after the hand grip exercises after four and eight weeks of exercise in patients with renal failure. However, the results were obtained through ultrasonography and the vein of interest was a superficial vein [16]. Similar findings are reported by Nantakool and co-researchers who reported an increase in the diameter of cephalic vein after six to eight weeks of isotonic and isometric exercises[19]. In our study venous diameter was not assessed however, luminal area was measured. Contradictory to our findings was a study by Anna Oue and coworkers which evaluated that after exercise regimen the luminal diameter of the superficial veins was increased however at same time there was a decrease in the deep veins[9]. These contradictory findings can be because of the difference of the vessels, since our research was conducted on the central larger veins namely superior and inferior vena cava, whereas in that study vessels of interest were superficial veins of the upper arm.

Another parameter evaluated in our study was wall to lumen ratio which was significantly modified in both the vessels of concern. As elaborated by Green et al. wall to lumen ratio may be affected by exercise. They elaborated that this alteration either might be the consequence of vessel wall thickness alterations or acute change in the said ratio it may be attributed to the contraction of the smooth muscles in the vessel wall[17,18]. This study was however conducted on the large conduit arteries yet giving an insight of the mechanisms involved in the alteration of the wall to lumen ratio. The confounding results of our study in particular demand further meticulous evaluation on the topic.

The novelty of the topic has been a great hindrance in comparison to the finding of our study with available literature. Yet, this can be ascertained that venous ultrastructure is greatly impacted by the exercise. It is very unfortunate that in the modern era of medicinal advancements we are still lacking comprehensive findings on a very vital component of cardiovascular system.

In this study only one type of the exercise was used, further the study was carried out on the large central veins. It is recommended for the future that more studies should be carried out using exercise modalities of different types and duration. In addition, smaller peripheral veins should also be evaluated.

Conclusion

It is concluded that the wall thickness and luminal area of both superior and inferior vena cava were significantly increased after intervention of moderate intensity continuous exercise. In addition, the wall to lumen ratio was also significantly altered after exercise in both large veins. Therefore, it can be concluded that the histological structure of veins shows adaptation to exercise as the other components of the cardiovascular system such as heart and arteries.

Conflict of interest

Authors have no conflict of interest and no grant / funding from any organizatio

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