2021

Association Between Antioxidant Capacity and Vascular Hemodynamics in Premenopausal Women Following 12 Weeks of Exercise Training

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ASSOCIATION BETWEEN ANTIOXIDANT CAPACITY AND VASCULAR HEMODYNAMICS IN PREMENOPAUSAL WOMEN FOLLOWING 12 WEEKS OF EXERCISE TRAINING

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A THESIS

Submitted to the graduate faculty of The University of Alabama at Birmingham,
in partial fulfillment of the requirements for the degree of
Master of Science

BIRMINGHAM, ALABAMA

2021

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KINESIOLOGY

ABSTRACT

Background: Oxidative stress induced by "free radicals" plays role in vascular dysfunction and cardiovascular health. Studies exploring beneficial effects of chronic aerobic training by enhancing antioxidant mechanisms in the body and thus, hemodynamic outcome measures are limited.

Purpose: To assess effects of chronic aerobic exercise training on association between change (Δ) in antioxidant capacity, measured in terms of ferric reducing ability of plasma (ΔFRAP), and change in vascular hemodynamic (outcome) measures: systolic blood pressure (ΔSBP), diastolic blood pressure (ΔDBP), large arterial elasticity index (ΔLAEI) and small arterial elasticity index (ΔSAEI), in a cohort of healthy women.

Methods: This was a secondary data analysis of a study designed to evaluate cardiometabolic outcomes. Participants performed 12-weeks of supervised aerobic exercise 3 times/week on stationary cycle ergometer. Change in FRAP and hemodynamic measures was calculated using baseline and post-training (wk 12) values.

Results: Analysis included 29 healthy females (African-Americans=15, Caucasians=14) with mean age of 32 years. Significant negative correlation between ΔFRAP and ΔDBP ($r=-0.48$, $p=0.01$) and positive correlation between ΔFRAP and ΔSAEI ($r=0.38$, $p=0.04$)

were observed. In multiple regression analyses, a model with ΔFRAP and ΔSAEI significantly predicted ΔDBP (p=0.02) with ΔFRAP (p=0.04) having an independent significant effect. In a model of ΔSBP although overall effect of ΔFRAP and ΔSAEI was significant (p=0.04), ΔFRAP (p=0.25) did not have significant independent effect.

Conclusion: When antioxidant capacity of plasma changes after exercise training, this may be related to the changes in diastolic blood pressure and small arterial elasticity.

Keywords: Aerobic exercise training, healthy subjects, blood pressure, arterial elasticity, oxidative stress, antioxidant capacity

ACKNOWLEDGMENTS

I would like to acknowledge my committee members for their valuable guidance and support without which this project could not have been completed.

I would like to extent my sincere thanks to Dr. Gordon Fisher, Dr. Gary Hunter and Dr. Douglas Moellering for their valuable contribution throughout the project.

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BP	blood pressure
DBP	diastolic blood pressure
FRAP	ferric reducing ability of plasma
LAE	large artery elasticity
LAEI	large artery elasticity index
LDL	low-density lipoprotein
NO	nitric oxide
OS	oxidative stress
RNS	reactive nitrogen species
ROS	reactive oxygen species
SAE	small artery elasticity
SAEI	small artery elasticity index
SBP	systolic blood pressure

INTRODUCTION

Interrelationship between pressure, flow and resistance in the closed loop of the circulatory system, is a key determinant of vascular hemodynamics. Flow rate of blood through the vascular system is proportional to the pressure difference across the system, but inversely proportional to the resistance [1]. A loss of arterial elasticity leading to increased vascular resistance increases the risk for cardiovascular events. Oxidative injury to the vessel wall may be one of the underlying mechanisms influencing arterial elasticity [2].

Reactive oxygen species (ROS) and reactive nitrogen species (RNS), collectively known as "free radicals", play role in oxidative injury to vessels. They are formed as a byproduct of normal aerobic metabolism and are signaling molecules that play important role in controlling various cellular functions. Excess free radicals are usually removed or inactivated in vivo by antioxidants, however, an accumulation of free radicals increases the potential of structural and functional damage to biologically important cellular components, such as DNA, proteins, lipid rich cell membranes; leading to altered cell signaling, impaired energy metabolism, and inflammation [3, 4]. This process of "oxidative stress", was defined by Dean Jones and Helmut Sies, as a state of "an imbalance between oxidants and antioxidants in favor of the oxidants, leading to redox signaling disruption and/or molecular damage" [5].

Oxidative stress is recognized to be involved in many physiological conditions such as aging and exercise. Electron leak in the mitochondria and alterations in the blood

flow during exercise, contribute to formation of free radicals [4]. Various pathological conditions; including diabetes mellitus, hypertension, obesity, peripheral arterial disease, chronic obstructive pulmonary disease, systemic lupus erythematosus, and cancer are associated with increased oxidative stress [6, 7].

ROS induced oxidation of lipids generates more stable and highly reactive lipid peroxidation products, such as oxidized low-density lipoproteins. Oxidized lipids can cause selective alterations in cell signaling. They can be cytotoxic in nature, initiating death of vascular smooth muscle cells, through necrosis and apoptosis [8, 9]. During phagocytosis, myeloperoxidase rreleased from cytoplasmic granules of neutrophils and monocytes, plays role in the LDL oxidation, leading to the pathogenesis of diseases, including initiation of atherosclerotic lesions in vascular endothelium [10, 11].

Hypertensive effects of oxidative stress are mainly due to endothelial dysfunction and impaired vascular tone. Degradation of nitric oxide (NO) by free radicals leading to reduced (NO) bioavailability in known to play role[12]. In addition, membrane-bound NADPH oxidase enzymatic system, which gets activated by Angiotensin II and aldosterone, is the major source of ROS causing vascular damage [13] .

The relationship between markers of oxidative stress, and arterial elastic properties and role of exercise in improving endothelial function has been studied in animals and human subjects. Role of oxidative stress in contributing to the hypertension, placental and liver damage was demonstrated in an animal model by Morris and colleagues [14]. Rocque et al. concluded that exercise training in spontaneously

hypertensive rats improved endothelial function and vascular stiffness in coronary and small mesenteric arteries, possibly due to the concomitant decrease of oxidative stress and increased NO bioavailability [15]. A study by Groussard et al. in zucker rats, showed that moderate-intensity continuous training (MICT) increased the antioxidant system marker (FRAP) in epididymal adipose tissue [16]. Although, vascular tissues from hypertensive animal models demonstrated increased oxidant burden, human studies are needed to convincingly demonstrate an association between oxidative stress and arterial stiffness [17]. Although underlying mechanisms of exercise mediated antihypertensive effects are not fully clarified, improvement of endothelial adaptation, mediated by significant increase in vascular NO production and/or decrease in NO scavenging, is one of the suggested mechanisms [18, 19].

Muscle contractions during exercise lead to formation of ROS. Although, moderate levels of ROS are essential for producing normal muscle force, excess levels cause oxidative damage leading to muscle fatigue [1, 20]. Endogenous sources of ROS include mitochondria, NADPH oxidase, xanthine oxidase, lipoxygenases and macrophages activated by muscle injuries due to strenuous exercise. Regular exercise prevents negative effects of ROS by increased expression of antioxidants [20, 21]. Exercise induced increase in the blood flow and laminar shear stress stimulate the antioxidant cascade at the level of the vascular endothelium. Steady or pulsatile laminar shear stress induces expression of genes encoding cytoprotective enzymes, which are regulated by the transcription factor nuclear factor (erythroid-derived 2)-like 2 (Nrf2)

[22]. Increasing evidence indicates that the Nrf2 pathway plays a key role in how oxidative stress mediates the beneficial effects of exercise.

A systematic review and meta-analysis of controlled trials conducted by de Sousa and colleagues revealed that regardless of intensity, volume, type of exercise, and studied population, the antioxidant measures tended to increase whereas pro-oxidant markers tended to decrease after training, and concluded that exercise training seemed to induce an antioxidant effect [23].

Individuals undergoing exercise training have high levels of antioxidant enzymes and certain non-enzymatic antioxidants in muscle and demonstrate greater resistance to exercise-induced or oxidative stress [24]. Bouts of acute exercise leads to episodic increases in oxidative stress. Further, repeated and regular exercise, leads to upregulation of endogenous antioxidant defenses, leading to overall greater ability to counteract the damaging effects of oxidative stress [25]. Previous studies have predominantly focused on the effects of regular exercise on the oxidative stress in patient population with cardiovascular conditions, or effects of maximal exercise in healthy participants, or in animal models. Therefore, the purpose of this study was assess the effects of 12 weeks of aerobic exercise training on changes in antioxidant capacity and vascular hemodynamic responses in a cohort of healthy women. We hypothesized that antioxidant capacity would be associated with changes in hemodynamic responses following exercise training.

METHODS

This is a secondary analysis of a study that was designed to evaluate insulin sensitivity, resting energy expenditure, and blood pressure following a bout of moderate-intensity or high-intensity exercise as compared with no exercise (no exercise for 72 hours before evaluation) [26, 27]. Out of total 49 participants enrolled in the original study, we included 29 participants who had data available for both ferric reducing ability of plasma (FRAP), an independent variable of interest, and vascular hemodynamic (outcome) measures viz. blood pressure (systolic and diastolic) and arterial elasticities (small and large).

Only the baseline and post-training evaluation (corresponding to the no acute exercise session i.e. trained state) were used for data analysis in this study. The other two conditions, either a bout of moderate-intensity or a bout of high-intensity cycle exercise 22 hours before evaluation were not considered in this study. Since in the original study, 3 postexercise training conditions [(1) no exercise within 72 hours, (2) following moderate-intensity exercise, and (3) following high-intensity exercise] were randomly assigned to avoid an order effect in analysis; the post-training evaluation following 72 hours of no exercise used in this study varied from 8 week to 16 weeks (average 12 weeks). No differences in change in maximum oxygen uptake were observed between the three training periods, i.e. 8 weeks vs 12 weeks vs 16 weeks.

Study participants

Female participants between 20 and 40 years of age participated in the original study. Participants had normal menstrual cycles, were not taking oral contraceptives or any medications known to influence glucose and/or lipid metabolism. Additional inclusion criteria included: 1) normotensive; 2) nonsmoker; 3) sedentary as defined by participating in any exercise related activities less than once per week; and 4) normoglycemic as evaluated by postprandial glucose response to a 75-g oral glucose tolerance test. All participants provided written informed consents prior to the study participation. Study procedures were approved by the Institutional Review Board at the University of Alabama at Birmingham [28].

Procedures

In the original study, participates were evaluated on total 4 occasions. First evaluation was considered baseline and involved initial screening in the untrained state. After baseline evaluations, all participants completed 12 weeks of supervised, aerobic training 3 times/week on a stationary cycle ergometer. Beginning at week 8, participants were evaluated week 12 and week 16; each separated by 1 month during follicular phase of the menstrual cycle. Participants stayed in a whole room respiration calorimeter for 23 hours before testing.

Supervised aerobic exercise training

After baseline testing, all participants aerobically exercise trained for the duration of the 16-week study. Continuous exercise on a cycle ergometer was performed 3 days per

week, beginning with 3-5 minute period for warm up and stretching at each session. During week 1, continuous exercise was maintained for 20 minutes at ≈67% of maximum heart rate. Over the first 4 weeks exercise intensity and duration increased so that by the beginning of the 5th week, exercise was maintained for 40 minutes at ≈80% maximum heart rate until the end of the study. Participants cooled down for 3-5 minutes, following cessation of exercise [26].

Room calorimeter

Participants spent 23 hours in a whole-room respiration calorimeter for measurement of total energy expenditure and resting energy expenditure before and after the exercise training period. Oxygen consumption and carbon dioxide production were continuously measured with the use of a magnetopneumatic differential oxygen analyzer (Magnos206; ABB, Frankfurt, Germany) and a nondispersive infrared industrial photometer differential carbon dioxide analyzer (Uras26, ABB). The calorimeter was calibrated before each participant entered the chamber. Prior to each test, calibration was carried out on the oxygen and carbon dioxide analyzers using standard gases. The full scale was set for 0%–1% for the carbon dioxide analyzer and 0%–2% for the oxygen analyzer. Each participant entered the calorimeter at 8:00 AM. Metabolic data were collected throughout the 23-h stay. Each participant was awakened at 6:30 AM the next morning in the calorimeter. Resting oxygen uptake was then measured for 30 min before the subject left the calorimeter at 7:00 AM [28].

Participants were provided with all food for 72 hours prior to tissue collection. We used multiple regression to develop equations designed to maintain energy balance before and during the room calorimeter stay. These equations have previously been used in the following papers (23, 25) Caloric intake for the 48 hours prior to the room calorimetry visit was based on estimates generated from 330 doubly labeled water estimates of free living energy expenditure of sedentary premenopausal women collected in our lab (26, 27). The first equation was: Equation 1 = 750 kcal + [(31.47 * fat-free mass) – (0.31 * fat mass) – (155 * race (race coded 1 for African American and 2 for European American)]. An equation for estimating the room calorimeter energy intake was developed from over 200 room calorimeter visits of premenopausal women (28, 29): Equation 2 = 465 kcal + [(27.8 * fat-free mass) – (2.4* fat mass) – (188*race (race coded 1 for African American and 2 for European American)]. However, we recognized that the estimates may result in overfeeding or underfeeding individual subjects. Therefore, we developed a correction equation for the room calorimeter visit that was based on energy expenditure during the room calorimeter stay up to 5:30 pm. This equation was: [Equation 3 = 9(390 kcal + average energy expenditure in kcal/min between 8:00 AM and 5:30 pm) * 925 kcal) – equation 2 estimate of energy expenditure]. We then adjusted the food intake of the evening meal to match the results of equation.

Blood pressure and arterial elasticity

Hemodynamic and arterial elasticity variables such as systolic blood pressure (SBP), diastolic blood pressure (DBP), large artery elasticity (LAE) and small artery

elasticity (SAE), were measured as reported previously [29]. These parameters were measured using noninvasive pulse wave analysis (HDI/Pulse Wave TM CR-2000, Hypertension Diagnostics, Eagan, MN). The arterial pulse wave analysis of the radial artery is based on a modified Windkessel model that allows evaluation of the large conduit arteries and the small microcirculatory arteries [30]. Briefly, with participants in the seated position, a solid-state pressure transducer array (tonometer) was placed over the radial artery of the dominant arm to record the pulse contour. The waveform was calibrated by the oscillometric method. Once a stable measurement was achieved, a 30 second analog tracing of the radial waveform was digitized at 200 samples per second. Before, during, and after the waveform assessment, an automated oscillatory BP measurement was taken on the contralateral arm. The first maximum wave-form observed represents the action of the arteries following cardiac ejection and reflects the large arteries, whereas the second rebound wave reflects compliance of the smaller arteries. SVR was calculated as the MAP divided by the ECO. Estimated cardiac index was calculated as the ECO divided by the body surface area. Total vascular impedance was determined from the modified Windkessel model evaluated at the frequency of the measured heart rate [31].

The ferric reducing ability of plasma (FRAP)

Total plasma antioxidant potential was determined by the FRAP assay according to the methodology of Benzie and Strain [32]. The basis of this assay is that water soluble reducing agents (antioxidants) in the plasma will reduce ferric ions to ferrous ions, which then react with an added chromogen. Working FRAP solution was prepared

daily and consisted of 300 mmol/L acetate buffer with the pH adjusted to 3.6 (3.1 g sodium acetate [Sigma, St. Louis, MO, USA] and 16 mL od 1N acetic acid [Sigma] per liter of buffer solution); 10 mmol/L 2,4,6-tripyridyl-s-triazine (TPTZ) [Sigma] in 40 mmol hydrochloric acid (HCl) [Fisher Scientific, Pittsburgh, PA, USA]; 20 mmol iron trichloride hexhydrate (ITX) [Sigma] in doubly distilled deionized water. Working FRAP reagent was prepared as required by mixing 25 mL acetate buffer, 2.5 mL ITX solution, and 2.5 mL TPTZ solution. The working FRAP solution was placed in a water bath and warmed to 37° C. Then, 10 μL of blank, samples, and ascorbate STDs were transferred by micropipette into designated well of 96 well plate. 300μL of FRAP reagent was then added to all wells containing blank, samples, and STDs. 96 well plate is then incubated for 4 minutes at 37° C before being read at 593 nm in a spectrophotometer. Samples and standards were analyzed in triplicate, and FRAP values were expressed as vitamin C equivalents as determined by linear regression from a vitamin C curve (0-1000 μmol) [32].

STATISTICAL ANALYSIS

All data were analyzed using IBM SPSS version 27. Descriptive statistics were computed at baseline and trained (post-training) state. All continuous variables were reported as mean and standard deviation (SD). Paired samples t-test was used to compare values at baseline and trained state. Relationship between change in FRAP (independent variable of interest) and change in vascular hemodynamic (outcome) measures at baseline versus trained state was assessed using Pearson's correlation (r) with coefficient of determination (r^2), and linear regression (univariate and multiple), and scatterplots. Based on the results of simple correlations, a multiple regression model was used to evaluate the effects of FRAP and SAEI on SBP and DBP. For all analyses, statistical significance was set at 0.05.

RESULTS

Total 49 female participants were enrolled in the original study. Total 29 participants who had both, baseline and post training FRAP, SBP, DBP, LAEI and SAEI values measured, were included for data analysis in this study. The ethnic composition of participants included in this data analysis was 14 Caucasians and 15 African-Americans. The mean age of the participants at baseline was 32.19 (SD=5.51) yrs.

Table 1 shows baseline, post-training characteristics, change between baseline and trained state (i.e., delta, Δ or d) and results of paired t-tests. Change (delta, Δ or d) was calculated as post training (12 weeks of training) minus Pre training (baseline) value of each variable. No significant differences were observed between baseline and post training values of any of the variables.

Table 1.

Baseline, post training, Δ and paired t test results

Characteristic	Baseline mean (SD)	Trained state or post-training mean (SD)	Delta (Δ) or (d)[a] Mean (SD)	p value
FRAP (μmol)	429.34 (74.29)	414.02 (84.64)	-15.32 (51.05)	0.12
SBP (mm Hg)	116.39 (11.42)	118.08 (12.26)	1.69 (9.00)	0.32
DBP (mm Hg)	69.30 (7.95)	70.29 (9.68)	0.99 (7.15)	0.46
LAEI (ml per mmHg x 10)	16.81 (4.81)	15.71 (4.70)	-1.10 (6.30)	0.36
SAEI (ml per mmHg x 100)	7.45 (2.32)	7.18 (2.32)	-0.27 (2.40)	0.55

DBP=Diastolic blood pressure, LAEI=Large artery elasticity index, SAEI= small artery elasticity index, SBP=Systolic blood pressure
[a]Change measured at baseline and post-training at week 12. Baseline values subtracted from those at the trained state

As shown in the table 2, Pearson's correlation showed a statistically significant negative correlation between ΔFRAP and ΔDBP (p=0.01). In addition, statistically significant positive correlation was found between ΔFRAP and ΔSAEI (p=0.04). Correlations between ΔSBP and ΔLAEI with ΔFRAP were not found to be statistically significant (p=0.06 and p=0.67, respectively) (Table 2).

Table 2.

Correlation for Δ FRAP and Δ vascular hemodynamic (outcome) measures

	Pearson's correlation	
Outcome measure	**Simple Correlation coefficient[b]**	**p-value[b]**
ΔSBP	-0.35	0.06
ΔDBP	-0.48	0.01*
ΔLAEI	-0.08	0.67
ΔSAEI	0.38	0.04*

DBP=Diastolic blood pressure, LAEI=Large artery elasticity index, SAEI= small artery elasticity index, SBP=Systolic blood pressure
[a]Change measured at baseline and post-training at week 12.
[b]Univariate linear regression.
* Significant at 0.05 level (2-tailed)
NOTE: The coefficients represent the change in FRAP for the respective outcome measure

Scatterplots showing association between ΔFRAP versus change in individual outcome measures (ΔSBP, ΔDBP, ΔLAEI and ΔSAEI) are presented below (Figures).

In multiple regression analyses, a model with ΔFRAP and ΔSAEI significantly predicted ΔDBP (p=0.02) with ΔFRAP (p=0.04) having an independent significant effect.

(Adjusted) r-square (coefficient of determination) increased from 20% to 22 %, when ΔSAEI was added to ΔFRAP (Table 3).

Similarly, in a model of ΔSBP as an outcome variable, although overall effect of ΔFRAP and ΔSAEI was found to be statistically significant (p=0.04), ΔFRAP (p=0.25) did not show statistically significant independent effect adjusting for ΔSAEI (Table 4).

***Table 3*.**

Model estimation for Δ diastolic blood pressure

	Model R	R^2	Slope	Standardized ***β***	Partial r	p-value
Intercept	0.522	0.273	-0.031	-		0.02[a]
ΔFRAP	-	-	-0.054	-0.389	-0.388	0.041
ΔSAEI	-	-	-0.687	-0.231	-0.242	0.214

[a]Model p value

***Table 4*.**

Model estimation for Δ systolic blood pressure

	Model R	R^2	Slope	Standardized ***β***	Partial r	p-value
Intercept	0.463	0.215	0.758	-		0.04[a]
ΔFRAP	-	-	-0.039	-0.222	-0.225	0.249
ΔSAEI	-	-	-1.239	-0.330	-0.326	0.091

[a]Model p value

DISCUSSION

The purpose of this study was to examine the association between change in antioxidant capacity and change in vascular hemodynamic measures following 12 weeks of aerobic exercise training, in healthy volunteers. We hypothesized that antioxidant capacity is associated with changes in hemodynamic responses following aerobic exercise training.

We found significant associations of ΔFRAP with ΔDBP, and ΔSAEI. ΔFRAP and ΔDBP were negatively associated while; ΔFRAP and ΔSAEI were positively associated. In addition, ΔFRAP showed independent effect on ΔDBP and increased percentage of variance observed when ΔSAEI was taken into account. Which suggests that, when antioxidant capacity of plasma changes after exercise training, this may be related to the changes in DBP and SAEI.

Blood pressure: In the past, studies examining effect of aerobic exercise on blood pressure have reported reductions in both, SBP and DBP, and supported use of such exercise for prevention of cardiovascular risk [33]. Meta-analysis showed reduced blood pressure after aerobic exercise, independent of intensity and number of exercise sessions [34], and considered aerobic exercise an effective intervention in the prevention and treatment of cardiovascular diseases via reduction in oxidative stress [35]. Although significant changes in post training blood pressure were not recorded in our group of normotensives, findings are in agreement with above, as we found the significant

negative association of ΔFRAP with ΔDBP. In addition, the ΔFRAP with ΔSBP correlation of – 0.35 approached significance (P = 0.06).

Arterial elasticity: Although effect of duration of aerobic exercise on arterial elasticity and cardiovascular hemodynamics was studied in the past, information available is limited and varies. Varying the length of moderate intensity aerobic exercise bouts not only affected arterial elasticity responses, but also demonstrated independent responses of large and small artery elasticities [36]. Moderate-intensity exercise transiently increased small arterial compliance but did not elicit more sustained increases in either large or small arterial compliance [37]. In contrast, another study observed no significant alterations in arterial stiffness after a bout of acute moderate intensity aerobic exercise, however, antioxidants increased [38]. Our findings are in line with results of above studies to some extent. We found that ΔFRAP and ΔSAEI significantly predicted change in ΔDBP. In addition, ΔFRAP showed independent effect on ΔDBP and increased percentage of that effect observed when ΔSAEI was taken into account. However, association between Δ LAEI with ΔFRAP could not be demonstrated. Findings from past studies show that the type of the exercise influences arterial elasticity and thus, therapeutic potential for blood pressure reduction. It is possible that the duration and intensity of the aerobic exercise used in our study was not adequate to elicit changes in larger arterial elasticity. In addition, our participants were assessed in energy balance and that may be one reason why FRAP and BP did not change after training.

Although aerobic exercise in known to favorably lower blood pressure, greater reduction effects were observed in patients with hypertension than those with normal blood pressure [39]. The subjects in our study were normotensive, decreases in blood pressure would be expected to be relatively small. In addition, the training stimulus may have been too low to achieve decreases in these normotensive women [26]. As volume of exercise, in terms of duration and intensity need to be taken into consideration to elicit pronounced changes in arterial elasticity adaptations, there is the possibility that type of the aerobic training in this study was not sufficient to elicit significant differences in arterial elasticities in baseline versus trained states.

Antioxidant profile: Aerobic training is known to improve redox balance [40]. Previous studies showed improved antioxidant profile after maximal exercise [41, 42]. Glutathione (GSH) antioxidant system may play role in promoting general adaptation to oxidative stress [43]. Based on this information, we anticipated to see improved antioxidant profile after exercise training. However, we did not find significant differences between baseline and post training FRAP. Findings reported previously found changes shortly after performance of maximal exercise [41, 42]. In our study, after 12 weeks of aerobic training, subjects did not perform maximal exercise and refrained from the exercise for 72 hours before testing. In addition, hypoxia and exercise associated dehydration leading to increased plasma osmolality were suggested as the contributing factors leading to oxidative stress responses to exercise [44, 45]. In addition, excessively high fat, carbohydrate diets are known to cause nutritional stress, affecting antioxidant

status [3]. In contrast, subjects in our study were in energy balance and presumably hydration balance. They all ate the same food, thus, possibility of nutritional effects on antioxidant profile can be ruled out.

The present study examined an association between change in the antioxidant capacity and hemodynamic responses following aerobic exercise training, when change was measured between baseline and trained states. In conclusion, change in the FRAP showed negative association with change in the diastolic blood pressure and positive association with change in the small arterial elasticity. Change in the FRAP and small arterial elasticity significantly predicted change in the diastolic blood pressure. In addition, change in the FRAP showed independent effect on change in the diastolic blood pressure. In conclusion, when antioxidant capacity of plasma changes after exercise training, this may be related to the changes in diastolic blood pressure and small arterial elasticity. Although our findings are encouraging, prospective studies with larger sample size are needed to evaluate effects aerobic exercise training on antioxidant profile and hemodynamic measures.

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SUPPLEMENTAL FIGURES

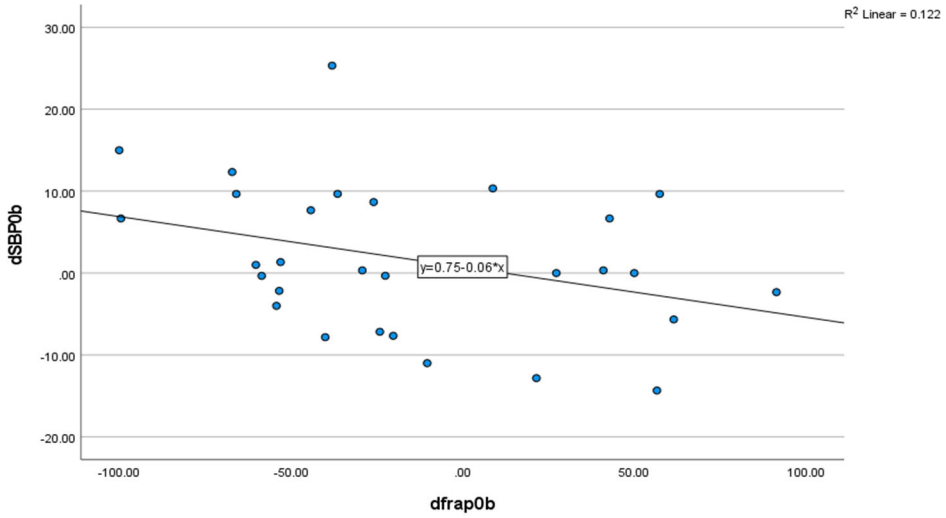

Figure 1. Scatterplot showing association between dFRAP and dSBP

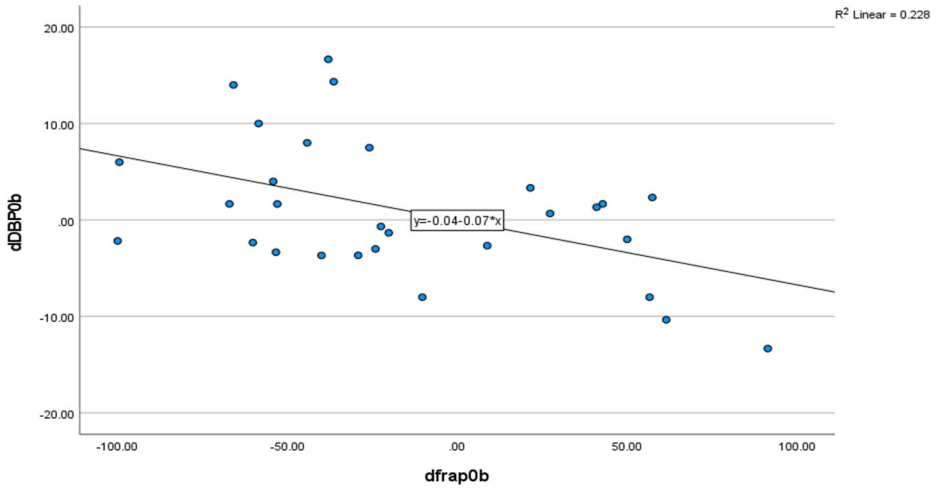

Figure 2. Scatterplot showing association between dFRAP and dDBP

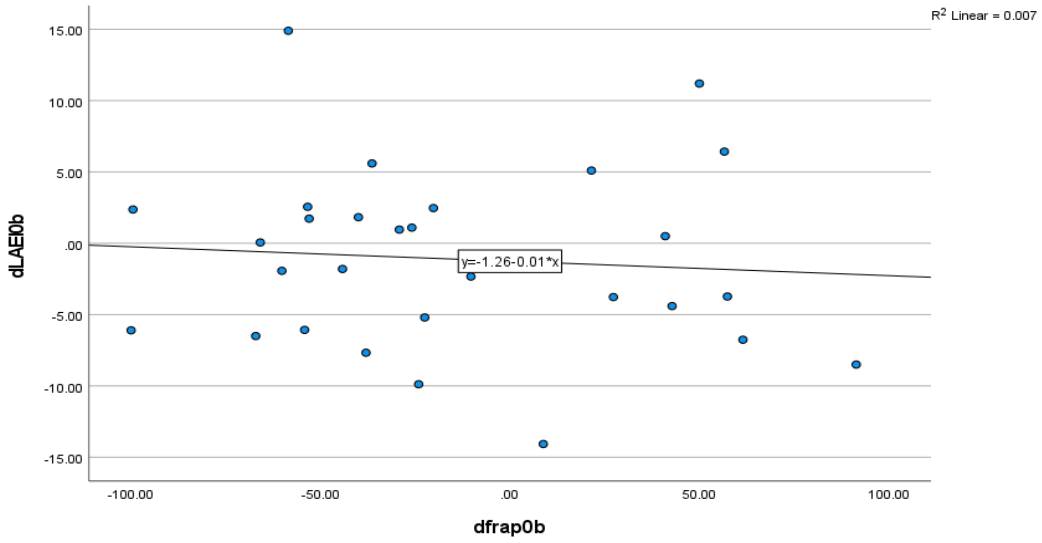

Figure 3. Scatterplot showing association between dFRAP and dLAEI

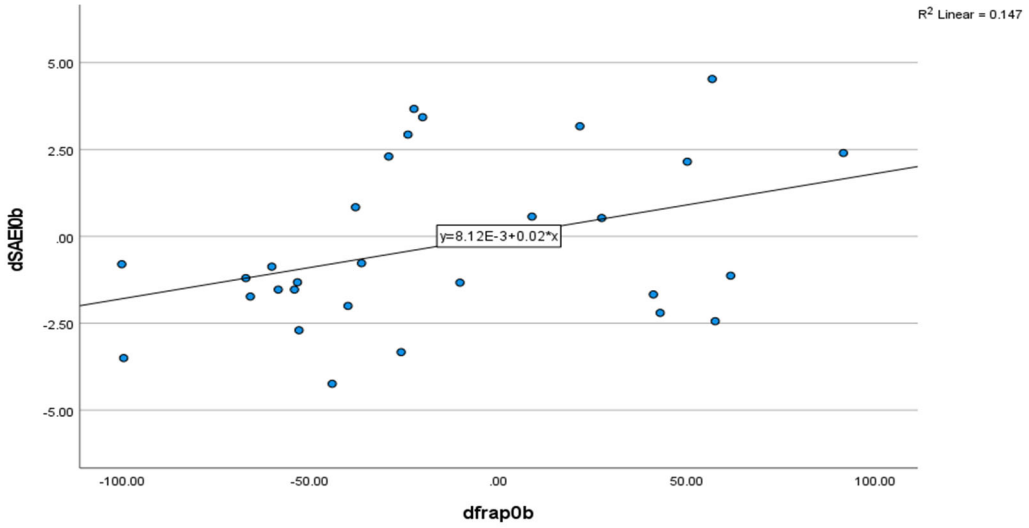

Figure 4. Scatterplot showing association between dFRAP and dSAEI

APPENDIX A

INSTITUTIONAL REVIEW BOARD (IRB) APPROVAL FORMS

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM
Office of the Institutional Review Board for Human Use

470 Administration Building
701 20th Street South
Birmingham, AL 35294-0104
205.934.3789 | Fax 205.934.1301 |
irb@uab.edu

APPROVAL LETTER

TO: Hunter, Gary R

FROM: University of Alabama at Birmingham Institutional Review Board
Federalwide Assurance # FWA00005960
IORG Registration # IRB00000196 (IRB 01)
IORG Registration # IRB00000726 (IRB 02)
IORG Registration # IRB00012550 (IRB 03)

DATE: 03-Nov-2020

RE: Exercise Intensity, Metabolic Rate & Insulin Sensitivity

The IRB reviewed and approved the Personnel Amendment submitted on 02-Nov-2020 for the above referenced project. The review was conducted in accordance with UAB's Assurance of Compliance approved by the Department of Health and Human Services.

Type of Review: Expedited
Expedited Categories: 8c,
Determination: Approved
Approval Date: 03-Nov-2020
Expiration Date: 02-Nov-2023

Although annual continuing review is not required for this project, the principal investigator is still responsible for (1) obtaining IRB approval for any modifications before implementing those changes except when necessary to eliminate apparent immediate hazards to the subject, and (2) submitting reportable problems to the IRB. Please see the IRB Guidebook for more information on these topics.

Documents Included in Review:

- IRB PERSONNEL EFORM

To access stamped consent/assent forms (full and expedited protocols only) and/or other approved documents:
1. Open your protocol in IRAP.
2. On the Submissions page, open the submission corresponding to this approval letter. NOTE:

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM
Office of the Institutional Review Board for Human Use

470 Administration Building
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irb@uab.edu

The Determination for the submission will be "Approved."
3. In the list of documents, select and download the desired approved documents. The stamped consent/assent form(s) will be listed with a category of Consent/Assent Document (CF, AF, Info Sheet, Phone Script, etc.)