

The comparison of endothelial function of moderate intensity interval exercise with continuous exercise in healthy men

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ABSTRACT

Background/aim: Exercise enhances endothelium-dependent vasodilation; however, it is unclear whether intermittent exercise has a different effect on vascular endothelial function compared to continuous exercise. This study aimed to compare vascular endothelial function following intermittent exercise including short rest intervals with continuous exercise, both at the anaerobic threshold level.

Materials and methods: Peak oxygen consumption (VO₂ peak) and anaerobic threshold were measured in physically active healthy young men (n = 12) by breath-by-breath analysis. After completion of intermittent exercise consisting of eight 1-min long intervals at the anaerobic threshold intensity with 75-s rest periods, total work was calculated. Equivalent work was done during continuous exercise. Immediately after the two exercise periods, venous blood lactate, endothelial nitric oxide synthase (eNOS), endothelin-1, N-terminal proANP (NTproANP), N-terminal proBNP (NTproBNP), and N-terminal proCNP (NTproCNP) levels were measured. Brachial artery flow-mediated dilatation (FMD) was measured before exercise and 30 min after exercise.

Results: Mean VO₂ peak level was 33.42 ± 5.9 ml/min/kg and anaerobic threshold level was 47.33 ± 5.85%. Lactate levels following continuous exercise were higher than levels following intermittent exercise (27.76 ± 7.43 mg/dl, 18.54 ± 4.87 mg/dl respectively; p < 0.05). Endothelin-1, eNOS, NTproANP, NTproBNP, and NTproCNP levels were similar after both modalities of exercise (p > 0.05). No significant difference was found in FMD response when comparing intermittent and continuous exercise (7.05 ± 15.11%, 2.49 ± 16.24% respectively; p > 0.05).

Conclusion: Since blood lactate levels are higher following continuous exercise, individuals who find difficulty exercising may prefer an intermittent form of exercise. However, both intermittent and continuous exercise at the anaerobic threshold level seem to not produce a significant acute change in endothelial function in healthy men.

1. Introduction

The effect of high-intensity exercise on endothelial function is more pronounced than the effect of moderate-intensity exercise (Ashor et al., 2015) however, significant improvements in endothelial function have also been reported after moderate-intensity exercise (Goto et al., 2003). On the other hand, interval-based exercise with short rest periods is suggested as a preferable option for improving health and fitness (Kilpatrick et al., 2015). A recent study reported that vascular and metabolic benefits of exercise are obtained properly through exercising willingly, but intensity above the anaerobic threshold level negatively affects

exercise adherence and reduces enjoyment (Ekkekakis et al., 2008).

In terms of endothelial function, previous studies have generally compared the effects of high-intensity interval exercise with moderate-intensity continuous exercise (Sawyer et al., 2016; Shenouda et al., 2017; Ramos et al., 2015). Ramos et al. (2015), reported that high-intensity intermittent exercise is associated with greater improvements in endothelial dysfunction compared to moderate-intensity continuous exercise. It is not clear whether moderate-intensity intermittent exercise and moderate-intensity continuous exercise have different effects on endothelial vascular function.

Nitric oxide (NO) plays an important role in the regulatory effect of

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exercise on vascular endothelial function (Fernandes et al., 2017). In addition, vasodilator natriuretic peptides and vasoconstrictor endothelial markers such as endothelin-1 are known potential biomarkers to evaluate endothelial function (Widmer and Lerman, 2014, McClean et al. (2015)). In addition to the blood based biomarkers, the flow-mediated dilatation (FMD) method is also commonly used for the evaluation of endothelial function (Thijssen et al., 2011). The findings of a study that applied the FMD method have shown that intense exercise caused higher shear stress and therefore more NO activation occurs (Ribeiro et al., 2010).

In this study, we aimed to compare the effect of moderate-intensity intermittent exercise at the anaerobic threshold level on vascular endothelial function with of continuous exercise. Since problems related to vascular dysfunction begin in the early stages of life (Bond et al., 2015), this study investigated brachial artery FMD responses and vasodilator or vasoconstrictor vascular endothelial markers in young men.

2. Material and methods

2.1. Study group

Twelve healthy young men aged between 18 and 24 years participated in this study. All participants stated that they had been doing more than 4 h of exercise per week for at least 1 year. All subjects had no medical history of any coronary heart disease, hypertension (i.e. resting blood pressure >140/90 mmHg), musculoskeletal diseases, or any pathology in electrocardiographic measurements (arrhythmias, long QT interval etc.) Subjects with regular drug use (antipsychotic drugs, glucocorticoids, bronchodilator drugs, etc.) were excluded. All participants were non-smokers for at least 6 months. The parents of the participants had no cardiovascular disease before the age of 55. Participants were informed of the risks associated with the study and written consent was obtained. Ethical approval of this study was approved by the Trakya University Faculty of Medicine Scientific Research Ethics Committee (TÜTF-BAEK, 2018/11) and the study procedures were carried out in accordance with the principles outlined in the Helsinki Declaration.

2.2. Study design

All participants visited the Trakya University exercise physiology laboratory three separate times separated by at least 24 h similar to a previous study (Mckay et al., 2009) within one month. Participants were instructed to continue recreational activities in addition to normal daily activities but to refrain from beginning any other training until the completion of the study. On the first visit, a physical examination was performed, anthropometric (height, weight) measurements were assessed, and fitness levels were evaluated using an athlete evaluation form by using a pretest questionnaire (Australian Sports Commission, 2000). Height was measured using a stadiometer (Seca 220, Hamburg, Germany), with subjects removing their shoes and standing close to the wall with their shoulders in a neutral position. Body weight, percentage of body fat, and lean body weight were measured with subjects wearing minimal clothing and no shoes using a bioimpedance analyzer (Tanita BC-418MA, Tokyo, Japan).

Body Mass Index (BMI) was calculated as weight in kilograms divided by height in meters squared. Resting blood pressure and heart rate were also measured at the first visit. A cardiopulmonary exercise test was also performed at the first visit to determine peak oxygen consumption (peak VO_2) and anaerobic threshold.

The subjects performed intermittent exercise on the second visit day. At the third visit, participants performed continuous exercise at an equivalent load to that done during intermittent exercise. The intensity of continuous and intermittent exercises was performed at the anaerobic threshold level, which had been calculated using peak VO_2 measurements. Blood samples were taken immediately before exercise, then 5

min and 60 min after the end of the exercise period; FMD assessments were performed for all participants before exercise and 30 min after exercise.

2.3. Measurements of peak oxygen consumption and anaerobic threshold

The peak VO_2 and anaerobic threshold levels of the subjects were measured breath-by-breath using a metabolic analyzer (Cortex, Metalyzer 3B-R2, Germany). An incremental cycling protocol of 60 revolutions per minute was used as described previously by Schaeffer et al. (2014) in which the workload was increased by 25 W every 2 min until exhaustion was reached on a cycle ergometer (Lode corival 906900, Groningen, the Netherlands). The test of peak VO_2 measurements lasted between 8 and 12 min. The anaerobic threshold was determined according to the V-slope method (Wasserman et al., 1990). The appropriate intensity of exercise was determined using the anaerobic threshold values calculated for each subject.

During the peak VO_2 measurements, the participants were monitored with a 12-lead electrocardiography monitor (Norav PC ECG 1200-HR-T, Wiesbaden, Germany). During the test, heart rate and blood pressure were regularly measured (every 3 min) with an exercise-adapted monitor (Tango Stress Test BP Monitor; Suntech Medical Instruments, Raleigh, NC). The humidity and temperature of the environment were recorded before each test and the atmospheric air in the gas analyzer was calibrated using 15% O_2 , 5% CO_2 mixed gas. Subjects were encouraged and motivated verbally during the exercise period.

Standard test termination criteria were used, as well as a decrease in the effort capacity of the subject. A plateau in oxygen uptake due to increased workload, a respiratory exchange ratio (RER) value above 1.1, and a heart rate at 90% of the predicted maximal heart rate (220 - age) levels were accepted as the criteria for terminating the test. All participants were expected to meet two of the three criteria above during the peak VO_2 measurement.

2.4. Moderate-intensity intermittent exercise

Moderate-intensity intermittent exercise was performed at the anaerobic threshold level using a cycle ergometer (Lode corival 906900, Groningen, the Netherlands) on the second visit. In a previous study on high-intensity intermittent exercise, an exercise period of eight 1-min intervals was studied (Bond et al., 2015). In this study, we used a similar protocol of exercise and rest intervals for moderate-intensity exercise. Thus, after a warm-up period of pedaling for 3 min against a 0 W load, participants were asked to perform eight 1-min intervals of exercise, with 75-s 20 W rest periods between each work interval. The participants rated perceived exertion using the Borg revised category-ratio scale (0–10 scale) immediately after exercise (Borg, 1990).

2.5. Moderate-intensity continuous exercise

In this study, moderate-intensity continuous exercise was performed at the anaerobic threshold level with an equivalent load to that done during intermittent exercise.

In order to determine the duration of the continuous exercise component for each subject, the work done during intermittent exercise was calculated (8 x watts at anaerobic threshold x time) and added to the work done during the rest intervals (7 x 20 W x time). Then, the total work done was divided by the anaerobic threshold value for each subject, i.e. [(8 x watts at anaerobic threshold x time) + (7 rest x 20 Watts x time)]/(watts at anaerobic threshold).

Participants were asked to pedal at the anaerobic threshold for the specified exercise duration. Similar to the intermittent exercise protocol, the moderate-intensity continuous exercise protocol started with a 3-min warm-up period with a 0 W load. The calculated minimum and maximum durations of continuous exercise were 9 min and 9 min 45 s

respectively (Fig. 1).

2.6. Flow-mediated dilatation assessment in the brachial artery

FMD measurements were performed using the 14 MHz superficial probe of a high-resolution ultrasonography device (Toshiba Aplio 500 Tokyo, Japan) in the brachial artery (Fig. 2).

Brachial artery was preferred for FMD assessment because it is a known as common site to represent endothelial-dependent functions in young adults (Pettersen et al., 2021).

FMD was measured pre-exercise and after 30 min following the end of the exercise periods.

Before each FMD measurement, participants rested for 20 min at room temperature (22–24 °C) to establish a hemodynamic steady state. The ultrasound probe was placed at the level of the cubital fossa to display the brachial artery in the longitudinal plane.

The grayscale and spectral Doppler images were obtained simultaneously and recorded on an external computer. For the spectral examination, the marker was placed in the middle of the vessel and the angle of insonation was set at 60°. All imaging procedures were performed on the right arm by the same blinded investigator. Standard guidelines were used to evaluate the endothelial function of the brachial artery via FMD (Thijssen et al., 2011; Corretti et al., 2002).

The baseline brachial arterial diameter was measured for 1 min. Following the baseline measurement, a pneumatic cuff was placed on the forearm and the blood flow to the brachial artery was occluded for 4 min by applying 220 mmHg pressure. After 30 s, image recording was started and continued for 2 min. The maximum percentage of increase above the baseline diameter (the average of records taken during the first minute) was calculated using an automatic edge detection system (FMD Studio system, Institute of Clinical Physiology, National Research Council, Pisa).

To calculate baseline diameter, measurements from the images obtained during the 1-min recording were averaged. The maximum diameter was calculated by averaging the diameter over the 0–90 s following the opening of the cuff (Black et al., 2008). FMD % [(maximum diameter - baseline diameter)/baseline diameter x 100] and FMD mm (maximum diameter - baseline diameter) were calculated as specified in the guidelines. The between-trial coefficient of variation for

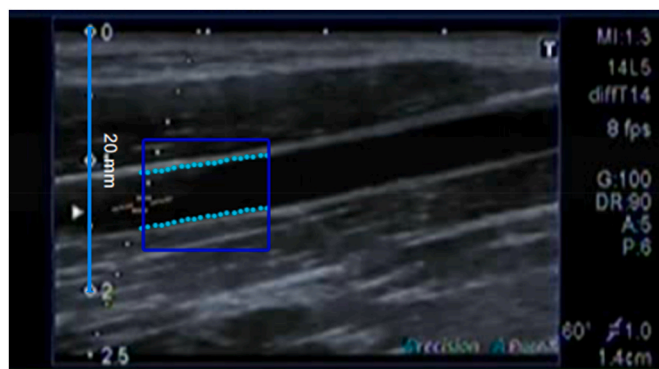


Fig. 2. The measurement of brachial artery diameter.

FMD was 6.3% (Gemignani et al., 2007, 2008).

All measurements were made at the same time of the day. Participants avoided consuming caffeine and alcohol for at least 8 h before the assessment, and avoided consuming high-fat foods for at least 6 h before the evaluation. In addition, all participants were asked about any recent vitamin or drug consumption. Since activation of the acute sympathetic nervous system may affect FMD, ultrasonic imaging was performed in a temperature-controlled, dim, quiet room in a supine position after the participants had rested for at least 20 min.

2.7. Blood sampling and analysis

At the first day of visit, a blood sample (10 ml) was taken from the antecubital arm vein of the arm. The blood sample was distributed into different tubes; a hemogram tube containing ethylenediaminetetraacetic acid for hematocrit analysis, another tube containing heparin for lactate analysis, and a dry tube for glucose level analysis. A tube containing a gel separator and coagulation activator was used to determine levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglycerides, cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL), potassium, and calcium. Blood samples were analyzed on the same day with the sample collection.

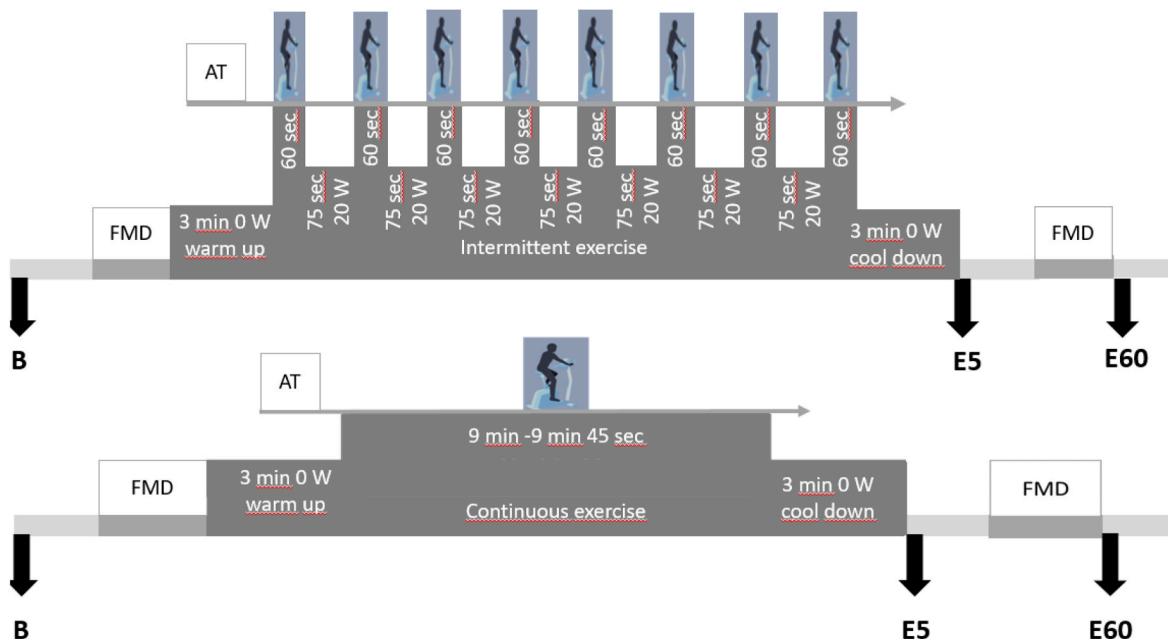


Fig. 1. Exercise protocol and measurements. B: Baseline; E5: Second blood sample in the 5th min after exercise; E60: Third blood sample 60 min after exercise; FMD: Flow-mediated dilatation. AT: Anaerobic threshold.

On the second and third days of the study, venous blood samples (5 ml) were collected through a cannula placed in the antecubital vein in order to explore endothelial function parameters. Venous blood samples were centrifuged at 3000 revolutions per minute for 15 min (MPW 350R, Poland). The serum was placed in Eppendorf tubes and stored at -80°C . Then, the enzyme-linked immunosorbent assay (ELISA) method was used for determination of serum concentrations of N-terminal proANP (NTproANP), N-terminal proBNP (NTproBNP), N-terminal proCNP (NTproCNP), endothelial nitric oxide synthase activity, endothelin 1 (ET-1), adiponectin, and leptin (Elabscience Cat. No: E-EL-H1848, Cat. No: E-EL-H0902, Cat. No: E-EL-H2538, Cat. No: E-EL-H0755, Cat. No: E-EL-H0064, Cat. No: E-EL-H0004, Cat. No: E-EL-H0113 respectively).

2.8. Statistical analysis

All data are presented as mean \pm standard deviation. Sample size was determined to be 12 based on adiponectin levels for moderate-intensity continuous and intermittent exercise ($d = 0.8$, $\alpha = 0.05$, and power 0.80). The normal distributions of NTproANP, NTproBNP, NTproCNP, eNOS, adiponectin, and leptin levels were examined using the One-sample Kolmogorov-Smirnov test. The Wilcoxon signed-rank test was used to compare FMD levels measured before and after exercise and the blood measurements taken at three different times. Statistical significance was determined as $p < 0.05$ in the study. The IBM SPSS software (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.) was used for analysis.

3. Results

In the study group, BMI levels were within the normal range; and, anthropometric and performance characteristics of the participants are shown in Table 1. The hematological measurements were within physiological limits (Table 2). For blood lactate levels, there was a statistically significant increase after continuous exercise compared to intermittent exercise ($p = 0.008$; Fig. 3). No statistically significant difference was found in the Borg score from the two exercise regimes (2.1 ± 0.8 vs 2.8 ± 1.1 ; $p = 0.058$).

The values of brachial artery diameter, peak artery diameter, FMD (mm), and % FMD values obtained before intermittent exercise did not differ from those obtained after exercise. Similarly, there was no significant difference when comparing these parameters before and after continuous exercise (Table 3).

The difference between FMD (mm) measurements taken before and after intermittent exercise (0.07 ± 0.60) was compared to the difference between measurements for continuous exercise (0.28 ± 0.68), with no significant difference shown ($p = 0.388$). Similarly, the change in FMD

Table 1 Anthropometric and performance characteristics of the participants.

Parameters	(n = 12) (Mean \pm SD)
Age (yrs)	22.0 \pm 1.5
Weight (kg)	71.9 \pm 8.6
Height (cm)	177.3 \pm 7.9
BMI (kg/cm ²)	22.8 \pm 1.8
Total body water (kg)	46.2 \pm 4.2
Fat free mass (kg)	63.2 \pm 5.8
Fat (%)	11.6 \pm 5.7
Peak VO ₂ (L/dk)	2.3 \pm 0.3
Peak VO ₂ (mL/dk/kg)	33.4 \pm 5.9
Peak heart rate (pulse per minute)	172.8 \pm 11.2
Peak heart rate (%)	86.2 \pm 6.0
Peak workload (W)	195.4 \pm 25.9
Anaerobic threshold (L/dk)	1.5 \pm 0.2
Anaerobic threshold (%)	47.3 \pm 5.8
Anaerobic threshold (W)	124.1 \pm 15.7

BMI: Body Mass Index; Peak VO₂: Peak Oxygen Consumption; W: Watt.

Table 2 Hematological characteristics of the participants.

Parameters	(n = 12) (Mean \pm SD)
Hemoglobin (gr/dl)	14.7 \pm 0.9
Hematocrit (%)	43.8 \pm 2.9
Erythrocyte (uL)	4.9 \pm 0.3
Leukocyte (uL)	6.2 \pm 1.0
Glucose (mg/dl)	84.0 \pm 8.7
Insulin (uIU/ml)	13.0 \pm 8.4
Cholesterol (mg/dl)	157.2 \pm 30.6
HDL (mg/dl)	45.8 \pm 11.1
LDL (mg/dl)	97.2 \pm 21.1
Triglyceride (mg/dl)	139.8 \pm 79.0
ALT (U/L)	20.1 \pm 12.8
AST (U/L)	21.5 \pm 7.2
Calcium (mg/dl)	9.6 \pm 0.5
Potassium (mmol/L)	4.3 \pm 0.5

HDL: High density lipoprotein; LDL: Low density lipoprotein; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

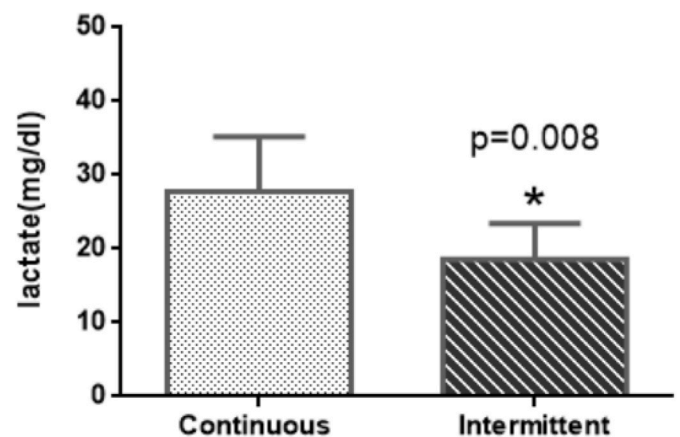


Fig. 3. Change in lactate level after continuous and intermittent exercise.

Table 3 Flow-mediated dilatation levels before and after intermittent and continuous exercise.

		Intermittent exercise	Continuous exercise	p
Baseline artery diameter (mm)	Before	3.95 \pm 0.32	4.04 \pm 0.39	0.358
	After	3.92 \pm 0.38 ^{NS}	3.98 \pm 0.38 ^{NS}	0.556
	Δ	-0.03 \pm 0.33	-0.01 \pm 0.35	0.583
	Change			
Peak artery diameter (mm)	Before	4.01 \pm 0.29	4.04 \pm 0.39	0.723
	After	3.95 \pm 0.35 ^{NS}	3.98 \pm 0.38 ^{NS}	0.272
	Δ	-0.06 \pm 0.28	-0.01 \pm 0.35	0.583
	Change			
FMD (mm)	Before	0.38 \pm 0.32	0.25 \pm 0.32	0.099
	After	0.45 \pm 0.53 ^{NS}	0.54 \pm 0.64 ^{NS}	0.638
	Δ	0.07 \pm 0.61	0.29 \pm 0.68	0.388
	Change			
FMD (%)	Before	9.62 \pm 7.57	6.26 \pm 7.99	0.136
	After	12.12 \pm 14.22 ^{NS}	13.32 \pm 14.29 ^{NS}	0.875
	Δ	2.50 \pm 16.24	7.06 \pm 15.11	0.388
	Change			

FMD: Flow-mediated dilatation. NS: Nonsignificant difference from before.

(%) value following intermittent exercise was not statistically different from the change following continuous exercise (2.49 ± 16.24 vs 7.05 ± 15.11 ; $p = 0.388$). There were also no statistically significant differences observed for eNOS activity, ET-1, adiponectin, leptin, NTproANP,

NTproBNP, and NTproCNP levels (Table 4).

4. Discussion

The findings of this study showed that a single episode of intermittent exercise at the anaerobic threshold level and continuous exercise had a similar effect on endothelial function in healthy men. On the other hand, lower blood lactate concentration observed after intermittent exercise; and, this difference in lactate level may facilitate individuals who struggle with being physically active to adhere to exercise.

The physical fitness levels of the subjects may be a factor that affects endothelial responses. According to the previous studies, the acute effect of a single bout of exercise on endothelial function may be affected by the physical fitness levels of individuals. For example, Phillips et al (Phillips et al., 2011). examined the effects of exercise on endothelial function in sedentary and physically active individuals. They reported that acute exercise did not significantly alter brachial artery % FMD in active individuals; however, there was a decrease in sedentary individuals. As a result, they suggested that sedentary subjects were more prone to impairment of endothelial function than physically active individuals following acute exercise. In addition, Harris et al. (2008) reported an exercise-induced acute decrease in FMD in sedentary individuals; but, an increase in active individuals. The present study conducted on physically active individuals who have vascular adaptation to exercise; thus, the vascular effects resulted from a sedentary lifestyle may not determine. Participants in our study were healthy men who exercised at least 4 days and a total of more than 4 h a week. The findings of our study show statistically nonsignificant increases in % FMD both in continuous and intermittent exercise, without any decrease in endothelial responses, in physically active healthy individuals.

The intensity of exercise may be another critical factor that affects endothelial response. According to the findings of a previous study, exercise at 50% of the VO₂ max can be defined as moderate exercise (Goto et al., 2003); therefore, in this study, participants performed exercise at the anaerobic threshold level (47.3 ± 5.8), an intensity that can be considered moderate (Goto et al., 2003). However, there are differences between individuals regarding anaerobic threshold levels. In this study, the anaerobic threshold of the participants varied between 36% and 54% of the VO₂ max. Our study took into account personal differences in metabolism: calculation of the anaerobic threshold level was the preferred method to determine the appropriate exercise intensity specifically for each person.

In our study, basal artery diameters and FMD levels (mm and %) were similar before and after exercise, with both interval and continuous exercise. This finding suggests that moderate intensity exercise, when applied intermittently or continuously, does not stimulate a vascular response affecting these parameters. In support of this, the present study showed that blood level of ET-1 as a potent vasoconstrictor peptide and eNOS activity as a vasodilator factor for NO synthesis did not change either 60 min after intermittent or continuous exercise.

These blood markers were measured at the same time points following exercise in studies (Numao et al., 2008; Kraemer et al., 2003; Wiecek et al., 2018; Boeno et al., 2019). In a previous study, McClean et al., 2015 also suggested that ET-1 concentration increases immediately after moderate-intensity treadmill exercise, with levels continuing to elevate for 3 h. Contrary to this finding, our study demonstrated no significant time-dependent change in ET-1 levels at least an hour following intermittent and continuous cycling exercise at moderate intensity. In terms of a vasodilatory response to exercise, it is known that

Endothelial-dependent vasodilation can be regulated natriuretic peptides in addition to NO (Widmer and Lerman, 2014). In our study, ANP, BNP and CNP were investigated in addition to eNOS levels in the blood; N-terminal forms of these hormones were measured to evaluate the more stable forms of natriuretic peptide hormones with longer half-lives. Our findings showed that levels of natriuretic peptides in the blood did not differ between intermittent and continuous exercises. This

Table 4
Comparison of vascular markers between intermittent and continuous exercise.

Parameters		Intermittent exercise (n = 12)	Continuous exercise (n = 12)	p
eNOS activity (pg/mL)	B	244.03 ± 185.30	309.00 ± 164.88	0.182
	E5	242.38 ± 203.03 ^{NS}	273.57 ± 144.90 ^{NS}	0.583
	E60	198.38 ± 139.61 ^{NS}	333.49 ± 205.01 ^{NS}	0.099
	Δ _{B-E5}	1.65 ± 128.69	35.43 ± 119.34	0.583
	Δ _{B - E60}	45.65 ± 67.31	-24.49 ± 141.43	0.099
	Δ _{E5 - E60}	44 ± 135.42	-59.92 ± 145.35	0.099
	Δ _{E60}			
Endothelin 1 (pg/mL)	B	16.76 ± 11.11	15.88 ± 10.30	0.583
	E5	20.80 ± 14.94 ^{NS}	17.76 ± 12.34 ^{NS}	0.248
	E60	20.66 ± 20.86 ^{NS}	15.88 ± 9.89 ^{NS}	0.155
	Δ _{B-E5}	-4.03 ± 5.41	-1.88 ± 5.54	0.182
	Δ _{B - E60}	-3.9 ± 12.18	0 ± 5.04	0.117
	Δ _{E5 - E60}	0.13 ± 10.16	1.87 ± 4.16	0.754
	Δ _{E60}			
Adiponectin (ng/mL)	B	212.05 ± 203.75	176.97 ± 102.42	0.814
	E5	180.32 ± 86.62 ^{NS}	211.37 ± 114.13 ^{NS}	0.347
	E60	165.69 ± 56.75 ^{NS}	182.58 ± 80.80 ^{NS}	0.272
	Δ _{B-E5}	31.73 ± 134.89	-34.4 ± 109.33	0.347
	Δ _{B - E60}	46.36 ± 173.01	-5.6 ± 97.27	0.272
	Δ _{E5 - E60}	14.64 ± 50	28.8 ± 105.11	0.583
	Δ _{E60}			
Leptin (pg/mL)	B	1013.73 ± 387.54	1557.26 ± 163.27	0.019
	E5	970.24 ± 1261.81 ^{NS}	1788.37 ± 2795.39 ^{NS}	0.272
	E60	1141.74 ± 1671.93 ^{NS}	1538.24 ± 478.21 ^{NS}	1.000
	Δ _{B-E5}	43.49 ± 397.74	-231.11 ± 711.81	0.272
	Δ _{B - E60}	-128.01 ± 396.97	19.02 ± 755.76	1.000
	Δ _{E5 - E60}	-171.5 ± 501.41	250.13 ± 570.94	0.308
	Δ _{E60}			
NTproANP (pg/mL)	B	1615.69 ± 797.20	1609.38 ± 848.71	0.754
	E5	1667.47 ± 866.37 ^{NS}	1612.61 ± 794.61 ^{NS}	0.583
	E60	1640.48 ± 832.58 ^{NS}	1523.71 ± 731.36 ^{NS}	0.530
	Δ _{B-E5}	-51.78 ± 148.84	-3.24 ± 215.51	0.583
	Δ _{B - E60}	-24.79 ± 272.74	85.67 ± 237.33	0.530
	Δ _{E5 - E60}	26.99 ± 289.48	88.91 ± 277.16	0.754
	Δ _{E60}			
NTproBNP (pg/mL)	B	591 ± 221,47	516,2 ± 88,84	0.272
	E5	567,7 ± 152,08 ^{NS}	569,4 ± 212,7 ^{NS}	0.158
	E60	559,7 ± 151,22 ^{NS}	584,6 ± 383,42 ^{NS}	0.937
	Δ _{B-E5}	0.02 ± 0.16	-0.05 ± 0.17	0.167
	Δ _{B - E60}	0.03 ± 0.11	-0.07 ± 0.36	0.875
	Δ _{E5 - E60}	0.01 ± 0.12	-0.01 ± 0.25	0.722
	Δ _{E60}			
NTproCNP (pg/mL)	B	333.88 ± 857.08	347.68 ± 858.57	0.158
	E5	311.09 ± 786.79 ^{NS}	297.39 ± 637.23 ^{NS}	0.347
	E60	302.05 ± 778.64 ^{NS}	332.55 ± 758.52 ^{NS}	0.209
	Δ _{B-E5}	22.78 ± 71.1	50.29 ± 223.55	0.374
	Δ _{B - E60}	31.83 ± 79.93	15.13 ± 105.93	0.209
	Δ _{E5 - E60}	9.05 ± 14.38	-35.16 ± 122.83	0.272
	Δ _{E60}			

B: Baseline; **E5:** 5th min after exercise; **E60:** 60 min after exercise. **NS:** Nonsignificant from Baseline.

finding was similar to the results of a recent study by Peres et al., which reported no difference between the effects of intermittent and continuous exercise on ANP and NO (Peres et al., 2018).

In the present study, moderate-intensity intermittent or continuous exercise did not cause a significant acute change in blood adiponectin which is known as a protective role on the cardiovascular system and endothelial function (Numao et al., 2008; Kraemer et al., 2003).

This finding thought that adiponectin appeared not to be a potent biological marker to evaluate moderate-exercise induced acute endothelial vascular responses. In addition, this study compared the change in blood leptin after intermittent and continuous exercise. According to our findings, a single episode of intermittent or continuous moderate-intensity exercise did not affect leptin levels in male volunteers. In contrast to our findings, Wiecek et al. (2018) reported that leptin levels in blood decreased in physically active women after a single bout of anaerobic exercise lasting 20 s. Our study did not aim to assess the effect of gender; however, it would be beneficial to investigate whether changes in blood leptin levels are dependent on gender in future studies. In this study, interval and continuous exercise bouts were performed at the same time of the day. At least 24 h' duration were allowed to the participants between the two exercise bouts. However, time intervals between interval and continuous tests could not be kept constant for all participants. Participants completed all the tests within one month that can be seen as a limitation.

On the other hand, lactate levels after intermittent exercise were lower than continuous exercises in our study. Supporting this finding, a previous study suggested that intermittent exercise causes less lactic acid accumulation by activating fewer anaerobic mechanisms in peripheral muscles and intracellular oxygen transport systems (Elander et al., 1985). The lower blood lactate levels associated with intermittent compared to continuous exercise may be an appealing factor that makes this type of exercise more agreeable for people who find exercising difficult.

In conclusion, the findings of our study show that a single episode of moderate-intensity intermittent exercise does not seem to have a different effect on vascular endothelial function compared to continuous exercise in physically active healthy individuals. However, our findings suggest that intermittent exercise may be less metabolically effective for causing fatigue and thus may be the preferred form of exercise for those hoping to adopt a physically active lifestyle.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Selma Arzu Vardar reports financial support and equipment, drugs, or supplies were provided by Trakya University.

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