Imaging Ultrasound Assessment of Exercise-Induced Endothelial Shear Stress of the Brachial and Carotid Arteries

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Endothelial shear stress (ESS) has a possible effect on regulation of gene expression in the protection against atherosclerosis. During exercise, ESS should increase as systolic blood pressure and heart rate (HR) increase too; however, it is hard to determine ESS changes during exercise. Imaging ultrasound assessment of the brachial and the carotid arterial blood flow during exercise might help to estimate exercise-induced ESS. We present here the methodology at the Clinical Applied Physiology Laboratory to estimate exercise-induced ESS. We normally perform 2 exercise tests in 2 different visits. First, a cardiopulmonary exercise test with serial microblood sampling to determine blood lactate (La) levels on a stationary cycle ergometer to determine maximal oxygen consumption, maximal exercising HR, and lactate threshold curve. The second exercise test includes three 5-min steady state stages determined by La levels from test 1 (La $<$2 mmol/L, La 2–4 mmol/L, and La $>$4 mmol/L). During the second test, we position an ultrasound probe holder on either the arm or neck to image the brachial or carotid arteries, respectively. We obtain images and blood flow velocities through Doppler at each exercise stage and then we analyze the images using edge detection software to determine artery diameters. With these data, we are able to estimate ESS, flow direction, and the presence of turbulent flow. **(Cardiopulm Phys Ther J. 2021;32: 30–36)** *Key Words: endothelial shear stress, exercise, carotid artery, brachial artery, ultrasound, lactate threshold*

INTRODUCTION AND PURPOSE

Cardiovascular diseases represent the first cause of death in the United States. It has been estimated that the total cost of cardiovascular diseases for the United States is approximately $316.1 billion, and according to 2030 projections, direct and indirect cost will reach $818 billion and $276 billion, respectively.[1] Risk factors associated to the development of cardiovascular diseases include high blood pressure, obesity, dyslipidemia, impaired fasting glucose, sedentary behavior, and physical inactivity.[2]

Exercise, a subtype of leisure-time physical activity, is a cornerstone strategy in the management of cardiovascular diseases, such as coronary artery disease, peripheral artery disease,[3] and stroke.[2] Initial reports attributed the benefits of exercise on cardiovascular health to a reduction in risk factors.[4] However, more contemporary epidemiological analyses revealed that the risk factor reduction obtained through exercise could only explain 40% to 50% of all the cardiovascular benefits, especially in coronary artery disease.[5,6] This risk factor gap implies that underlying exercise-induced molecular mechanisms may have an important role on cardiovascular protection.[4,5]

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Vascular homeostasis is closely regulated by the mechanical interaction between blood flow and endothelial cells, which is known as endothelial shear stress (ESS).[7] During exercise, both heart rate (HR) and systolic blood pressure increase in an intensity-dependent manner to match blood flow to metabolic demands.[8,9] This increased blood flow would increase pulsatility and ESS inside the arteries.[5,10,11] It has been proposed that these hemodynamic changes might regulate the expression of genes involved in the protection against atherosclerosis through a mechanotransduction pathway.[12] Although in vitro studies have shown that increased ESS impairs the expression of proatherogenic genes and favor the expression of antiatherogenic genes,[13–16] most of these experiments do not account for the pulsatile nature of blood flow and ESS observed in in vivo exercise models, such as the brachial and carotid arteries.[7,11]

The main purpose of the current study is to describe a standardized protocol for the imaging ultrasound assessment of brachial and carotid arterial blood flows during hyperemic conditions (ie, exercise) and the steps required to estimate exercise-induced ESS for pulsatile blood flow. As this standardized protocol uses exercise intensity as independent variable, a secondary purpose of the current study is to feature the use of blood lactate (BLa) levels as a clinical marker for exercise intensity. This methodology might help in reducing intratester and intertester variability in vascular ultrasound image acquisition during steady state exercise and will provide exercise-induced ESS reference values for translational clinical research.

METHODS

Participants

This study was approved by the Institutional Review Board of The University of Texas at El Paso and conformed to the Declaration of Helsinki. All participants provided written informed consent before their participation in the study. Twenty-four young, apparently healthy participants (14 women and 10 men, age range 18–35 years) were recruited from the Paso del Norte region. Participants were required to abstain from food, caffeine, alcohol, nonsteroidal anti-inflammatory drugs, and antioxidant supplementation for at least 8 hour before testing, and 24-hour abstinence from exercise. Premenopausal female participants were tested within 4 days before and after menses to standardize any hormonal influence on vascular reactivity.[17]

Protocol

The current protocol has been previously described.[18,19] Briefly, 2 exercise tests were performed on a cycle ergometer (Corival, Lode, The Netherlands) at the same time of the day, at least 48 hours apart. Before each exercise test, height (Seca Medical, Germany) and weight (WB-110A Class III, Tanita, Japan) were obtained. Seat height was adjusted to allow a $-5°$ to $-15°$ of knee extension during cycling. Participants were instructed to sit quietly on the bike for 10 minutes to ensure that increased sympathetic activity because of nervousness did not alter blood pressure readings.[20] During the resting period, 3 peripheral blood pressure values were recorded using an automated brachial blood pressure cuff (BP760, Omnron Healthcare, Inc., Lake Forest, IL).

The first exercise test was an 8 to 12 minute maximal, incremental cardiopulmonary exercise test that followed the American Heart Association and American College of Sports Medicine guidelines for exercise testing.[21,22] The first exercise test was designed to obtain maximal oxygen consumption (VO_2max), maximal exercising HR (HRmax), and lactate (La) threshold, which were used to determine exercise intensities for the second test. Blood La levels, assessed using microsample from the earlobe and analyzed with an automated lactate analyzer (Lactate Plus, Nova Inc., Boston, MA), HR (Quinton Q-Stress Cardiac Stress System, Mortara Instrument, Milwaukee, WI), VO_2 (TrueOne 2400, Parvomedics Inc., Sandy, UT), and rate of perceived exertion (RPE) (the Borg scale) were measured at rest and at the end of each 2-minute stage of the exercise test. Lactate threshold curves obtained during this first exercise test were used to determine the 3 steady-state workloads to be used for the second exercise test.

Before the second exercise test, 2 blood samples from the earlobe using microhematocrit capillary tubes were collected to perform centrifugation and determine hematocrit (HemataStat II Hematocrit Analyzer; Separation Technology Inc.), which will be used to estimate plasma dynamic viscosity for ESS calculations (see formulas before data analysis). Then, participants were asked to exercise 5 minute at each different workload, according to the following La training zones: low intensity (BLa of 0–2 mmol/L), moderate intensity (BLa of 2–4 mmol/L), and high intensity (BLa of >4 mmol/L).[23,24] Heart rate and VO_2 were continuously monitored throughout the 15-minute exercise test, as presented above, and BLa and RPE were assessed at baseline and at minutes 2 and 4 of each 3 exercise intensities.

During the second exercise test, a 12 to 18 Hz ultrasound transducer (LA435, Esaote, Firenze, Italy) was placed with its probe holder (patent pending[25]) on the right arm of the participant, 5 cm proximal to the antecubital fossa, or in a cervical probe holder around the neck of the participant to measure brachial and carotid arteries blood flow patterns, respectively (Fig. 1). During brachial artery measurements, the right arm was placed on a flat surface, maintaining an ~80° shoulder abduction with ~35 to 45° of horizontal flexion (Fig. 1A). In addition, the electrocardiographic signal was connected to the high-definition Doppler ultrasound machine (MyLab30 Gold Cardiovascular, Esaote, Firenze, Italy) and to an electrocardiogram trigger system (MP150WSW, BIOPAC Systems Inc., Goleta, CA and Frame Grabbing and Digital Data Input modules, Medical Imaging Applications LLC, Coralville IA) to obtain artery diameters, blood flow velocity, and flow direction. Analysis of

these variables was performed using automated edge-detection software (Vascular Research Tools, Medical Imaging Applications LLC).

Ultrasound settings were adjusted to 12 Hz of frequency and 3 cm of depth and to 12 to 18 Hz of frequency and 3 to 5 cm of depth for the imaging of the brachial and common carotid arteries, respectively. In both arteries, the transverse section of the artery was identified and centered. Then, the transducer was rotated 90° to obtain a longitudinal view of the vessel, and pulse wave Doppler was used to record the velocity of blood flow, ensuring an insonation angle <60° (Fig. 2). For the carotid artery, the pulse wave Doppler was placed 5 to 10 mm distal to the bifurcation to record blood flow velocity of the common carotid artery. Furthermore, blood flow velocities of the internal and external carotid arteries can be recorded if required by moving the pulse wave Doppler 5 to 10 mm proximally to the bifurcation. Once all the ultrasound settings are optimized, the transducer inside the probe holder was secured to limit any undesired movement of the ultrasound probe.

Endothelial shear stress was estimated during rest and each exercise bout by the Womersley approximation, using ESS = $\mu \times SR$ and SR = $2K \times V/D$, where μ is blood viscosity, SR is shear rate, V is peak systolic velocity, D is artery diameter, and K is a complex factor dependent only on the Womersley parameter α {$\alpha = (D/2) \times (\omega/[\mu/\rho])^{1/2}$, where D is artery diameter, ω is the angular frequency of the flow pulsation (ω = freq $\times 2\pi$), ρ is blood density, and μ is blood viscosity}.[7,11] Endothelial shear stress is expressed in dynes/cm.^{2} Blood viscosity and density were calculated using the following formulas[11,26,27]:

$$\mu_{plasma} = \frac{\exp\left[-5.64 + \frac{1800}{T+273}\right]}{SR},$$

$$\mu = \mu_{plasma} \times \exp(2.31HCT), \text{ and}$$

$$\rho = [1.09HCT + 1.035 \times (1 - \text{Hematocrit})]$$

where μ_{plasma} is plasma dynamic viscosity expressed in Pa·s, T is temperature expressed in °C, and HCT is the hematocrit expressed as a fraction.

Data Analysis

Descriptive statistics, including mean and SDs, were obtained. Normal distribution for all dependent variables was evaluated by the Kolmogorov–Smirnov test. The assumption of sphericity was checked by the Mauchly test and corrected by the Greenhouse–Geisser method when it was necessary. A one-way repeated measures analysis of variance with Fisher's least significant difference (LSD) pairwise comparison was used to detect differences in ESS between exercise intensities in both brachial and carotid arteries. The statistical analysis was performed with SPSS (version 24.0, IBM, Chicago, IL) and significance was set at $P < .05$.

RESULTS

All data were confirmed to be normally distributed. All data are presented as mean (SD). Demographic characteristics from the participants are shown in Table 1. Table 2 presents the exertional variables during each steady-state stage from exercise test 2.

Table 3 presents ESS in both brachial and carotid arteries at rest and during all 3 exercise intensities. In the brachial artery, exercise-induced ESS is intensity dependent in both antegrade and retrograde flows (Fig. 3). Similarly, exercise-induced ESS is intensity dependent in the common carotid artery (Fig. 4). Only antegrade flow was observed in the carotid artery.

DISCUSSION

Exercise-induced ESS has been proposed as a regulatory pathway of endothelial function. In vitro studies involving steady flow have shown that increased ESS upregulates endothelial nitric oxide synthase (eNOS) expression, an antiatherogenic gene that increases nitric oxide bioavailability, improving endothelial function.[28,29] However, most experiments are not based on realistic in vivo models, in which the pulsatile nature of blood flow is accounted to estimate ESS.[7] The purpose of the current study was to describe a standardized protocol for the imaging ultrasound

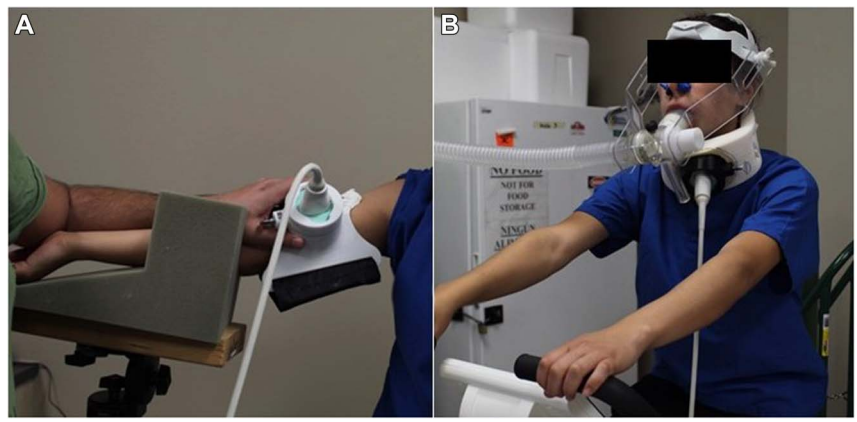

Fig. 1. Ultrasound imaging during exercise on the brachial artery (A) and carotid artery (B) using a patent pending probe holder.

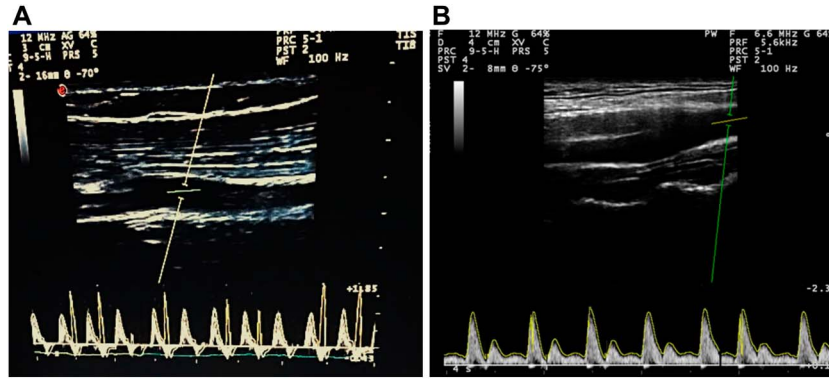

Fig. 2. Ultrasound and Doppler images of the brachial artery (A) and carotid artery (B) during exercise.

assessment of brachial and carotid arterial blood flows and to estimate ESS during exercise. The protocol described in this study allowed to estimate ESS in the brachial and carotid arteries, and the results showed that exercise-induced ESS is intensity dependent in both arteries.

In this protocol, we describe in depth all the necessary steps to calculate ESS from the brachial and carotid artery during an exercise test. For this purpose, live imaging of each of these vessels is obtained through Doppler ultrasound and recorded in edge-detection software. For the brachial artery, the position of the ultrasound transducer should be standardized to a specific distance from the antecubital fossa. In this protocol, we place the transducer 5 cm proximal to the antecubital fossa. It should be noticed that a probe holder (patent pending[25]) helps in the stability of the image, especially during exercise, where participants tend to move their arms and/or trunk (Figs. 1A, 2A). For the carotid artery, a probe holder is also recommended to maintain high-quality images during exercise (Figs. 1B, 2B). In addition, the Clinical Applied Physiology Laboratory has standardized the image acquisition and analysis during flow-mediated dilation showing good intertester and intratester reliability.[30] Both protocols, the present exercise testing and the image acquisition and analysis, may help in reducing intratester and intertester variability in vascular ultrasound image acquisition during steady-state exercise.

TABLE 1

Demographic Characteristics of the Sample

	Overall
N	24 (14 females)
Age (yrs)	22.9 (3.8)
Height (cm)	166.6 (8.2)
Weight (kg)	73.3 (12.8)
BMI (kg/m^2)	26.4 (4.1)
SBP (mm Hg)	104.5 (8.3)
DBP (mm Hg)	66.0 (5.4)
HR (bpm)	66.6 (8.2)
Hematocrit (%)	47.1 (2.8)
VO2max (ml/kg/min)	30.9 (5.9)

Data are presented as mean (SD).

BMI, body mass index; DBP, diastolic blood pressure; HR, heart rate; SBP, systolic blood pressure; VO_2max, maximum oxygen uptake.

The blood flow patterns observed in this study are similar to the ones previously reported.[11,19,31–33] For example, Gurovich and Braith[11] and Coovert et al.[19] found that exercise-induced ESS in the brachial artery was intensity dependent and bidirectional (ie, antegrade and retrograde). In addition, Jiang et al.,[31] Hellstrom et al.,[32] and Sato et al.[33] all found an increase in carotid artery blood flow with exercise intensity and, similarly to the results of this study, no retrograde flow during exercise. To the best of our knowledge, this is the first study showing estimations of ESS in vivo in the carotid artery during exercise. Interestingly, antegrade ESS in both brachial and carotid artery were similar at each exercise intensity (Table 3 and Figs. 3 and 4). These results are relevant as in vitro studies should use in vivo data to determine molecular and/or genetic changes at the cellular level, which is not always the case.[12,34,35] For example, Davies et al used 1 to 15 dynes/cm^2 to investigate changes in pinocytosis in bovine aortic endothelial cells,[34] and DePaola et al. used 25 dynes/cm^2 to investigate cell density and spatial distribution in also bovine aortic endothelial cells.[35] These 2 classical studies fell short to the in vivo data presented in this study; moreover, these in vitro values do not even match resting conditions (Table 3). Applying higher ESS to in vitro models might change some of the paradigm in exercise–induced ESS and the direct benefits of exercise in the vasculature. Following this paradigm, we have recently used the in vivo results of this study to design in vitro experiments and perform what we call "reverse translation" studies.[36–38] The results on these pilot studies have shown an intensity-dependent pattern in gene expression of adhesion molecules [eg vascular cell adhesion protein 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1)], oxidative stress, eNOS, and superoxide dismutase, where higher intensities are associated with larger antiatherogenic changes.[36–38]

Another novel element on the exercise test presented here is the use of BLa levels as a marker of exercise intensity. Blood La levels have been used as a biomarker for

TABLE 2

Exertion Variables Measured at Rest and During Steady-State Exercise at Low, Moderate, and High Intensity

	Rest	Low-Intensity Exercise (BLa 0–2 mmol/L)	Moderate-Intensity Exercise (BLa 2–4 mmol/L)	High -Intensity Exercise (BLa >4 mmol/L)	RM-ANOVA Effect Size (Partial Eta Squared)
Power output (W)	0.0 (0.0)	54.2 (28.3)[a]	91.5 (31.6)[ab]	126.2 (39.2)[abc]	0.888
Heart rate (bpm)	75 (12)	108 (17)[a]	138 (20)[ab]	161 (15)[abc]	0.890
Lactate (mmol/L)	0.9 (0.3)	1.4 (0.4)[a]	2.9 (0.6)[ab]	4.9 (0.8)[abc]	0.923
RPE (6–20)	6.0 (0.0)	8.2 (1.8)[a]	10.8 (2.0)[ab]	13.8 (2.2)[abc]	0.883
VO2 (ml/kg/min)	3.7 (0.8)	14.2 (4.6)[a]	20.3 (5.3)[ab]	26.8 (6.6)[abc]	0.904

Data are presented as mean (SD).

[a]$P < .05$ versus rest.

[b]$P < .05$ versus low intensity.

[c]$P < .05$ versus moderate intensity.

BLa, blood lactate levels; RM-ANOVA, repeated measures analysis of variance; RPE, rate of perceived exertion; VO_2, oxygen uptake.

exercise intensity and training monitoring in athletes for several years,[39] and recently, they have been proposed as intensity markers for exercise prescription in clinical population.[19,24] Blood La has been a recurrent physiology topic for the past 200 years; however, only since the mid-'80s La has been strongly associated with exercise intensity.[40–43] In fact, Brooks et al. showed that muscle-produced lactate was not a glycolysis final metabolic product; in fact, La can be metabolized inside the mitochondria to produce further energy as ATP.[42–45] These findings changed the way BLa levels were interpreted, using them now as an accurate tool to determine exercise intensity.[46–48] In fact, a recent study has confirmed that exercise intensity at the La threshold, measured by actual workload rather than a percentage in maximal oxygen uptake, is one of the best predictors of aerobic performance.[49] Latest improvements in technology have allowed researchers to determine BLa concentrations inexpensively with microsampling from a fingertip or earlobe, using less than 25 microliters of whole blood.[50]

TABLE 3

Endothelial Shear Stress in the Brachial and Carotid Arteries at Rest and During Steady-State Exercise at Low, Moderate, and High Intensity

	Rest	Low-Intensity Exercise (BLa 0–2 mmol/L)	Moderate-Intensity Exercise (BLa 2–4 mmol/L)	High-Intensity Exercise (BLa >4 mmol/L)	RM-ANOVA Effect Size (Partial Eta Squared)
Brachial artery antegrade ESS (dynes/cm^2)	43.9 (18.5)	55.8 (21.7)[a]	69.9 (25.6)[ab]	82.8 (30.3)[abc]	0.834
Brachial artery retrograde ESS (dynes/cm^2)	10.8 (6.6)	19.4 (10.3)[a]	24.9 (9.7)[ab]	32.2 (12.3)[abc]	0.816
Carotid artery antegrade ESS (dynes/cm^2)	42.7 (15.5)	54.8 (17.5)[a]	69.7 (21.0)[ab]	82.9 (25.2)[abc]	0.834

Data are presented as mean (SD).

[a]$P < .05$ versus rest.

[b]$P < .05$ versus low intensity.

[c]$P < .05$ versus moderate intensity.

BLa, blood lactate levels; ESS, endothelial shear stress; RM-ANOVA, repeated measures analysis of variance.

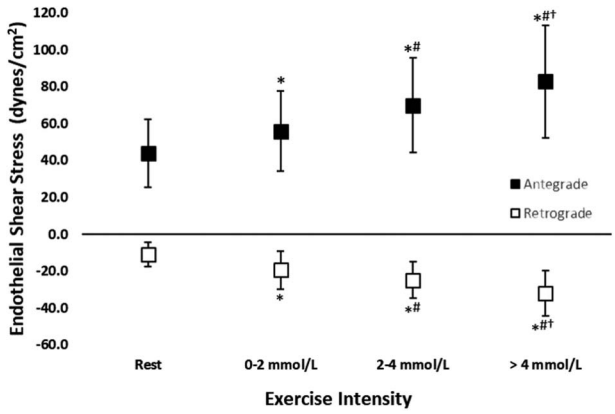

Fig. 3. Brachial artery exercise-induced endothelial shear stress. Data are presented as mean and error bars are SDs. Closed boxes: antegrade flow; open boxes: retrograde flow; *$P < .05$ versus rest; +$P < .05$ versus low intensity, #$P < .05$ versus moderate intensity.

These devices allow performing serial measurements during exercise, such as a graded exercise test, to determine the anaerobic threshold, which is defined as the exercise intensity when the La/intensity curve changes from a linear to an exponential relationship.[46,50–52]

Overall, the protocol described in this study showed that ESS is exercise-intensity dependent. If ESS is the main physiological factor for endothelial homeostasis and the lack of it is atherogenic,[7,12–16] exercise prescription for patients with coronary artery disease and stroke should consider the exercise intensities that produced the larger physiological effects. It is reasonable to say that performing this protocol is more realistic in research laboratories; however, clinicians might be able to use this information as evidence of (1) larger ESS is obtained at higher intensities and (2) larger ESS might be more appropriate to prevent, stop, and even revert, atherosclerosis progression.

This study was not without limitations. First, our sample was a convenience sample of young, healthy, college students. Having older participants or patients with coronary artery disease or stroke would have improved the reach of the current results to other populations. Our group has been working on clinical populations and the preliminary results show similar outcomes. Second, assessing blood flow patterns in the coronary arteries would have been ideal; however, the brachial artery shows a good replacement because it is a conduit muscular artery that has bidirectional flow as observed in the coronary circulation.[53] Finally, the use of BLa as a marker of exercise intensity is not regularly implemented and it is hard to interpret. Fortunately, technology has decreased the cost of BLa analysis, and it has started to be recognized as a potential therapeutic tool.[54]

In summary, the results of this study showed that (1) blood flow patterns during exercise can be determined in vivo, (2) exercise-induced ESS in vivo is exercise intensity dependent, and (3) the use of BLa levels as an exercise intensity marker could be considered when prescribing exercise after an exercise test.

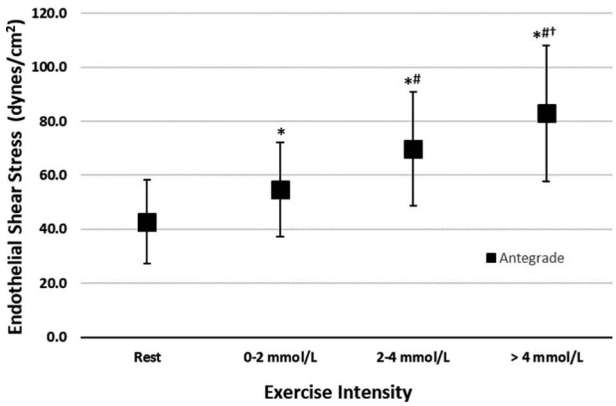

Fig. 4. Common carotid artery exercise-induced endothelial shear stress. Data are presented as mean and error bars are SDs. *$P < .05$ versus rest; +$P < .05$ versus low intensity, #$P < .05$ versus moderate intensity.

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