Differences in Blood Flow Patterns and Wall Shear Stress at the Carotid Artery Using Different Exercise Modalities and Intensities.

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# Abstract

# Introduction

Cardiovascular (CV) diseases, including coronary artery disease and stroke, are the leading cause of death rates in United States. One in every 19 deaths are produced by a stroke, and there are more than 610,000 new cases of stroke per year (Virani et al., 2021). Total direct and indirect cost of stroke for the country are estimated to be around 34 billion dollars(Benjamin et al., 2017). Atherosclerosis is responsible of 9 in every 10 cases of Stroke(Qaja & Bhimji, 2017); in addition, CV comorbidities are common features among stroke survivors(Tang et al., 2009). Endothelial dysfunction is recognized as the first step for the development of almost all CV diseases(Gurovich et al., 2014), and is a pathological condition characterized by an unbalance between vasodilatory and vasoconstrictory mechanisms (Flammer et al., 2012), and generally defined as the decrease in nitric oxide (NO) bio-availability within the endothelium(Harris et al., 2010). An underlying mechanism that regulates endothelial function is shear stress (SS), which is the frictional force produced between blood flow and endothelial cells(Sriram et al., 2016); where increments of SS (e.g. during exercise) are known to improve endothelial nitric oxide synthase (eNOS) gene expression (Ishibazawa et al., 2011). Exercise programs are one of the best suited approaches to prevent CV comorbidities and a subsequent stroke (Jurczak et al., 2014; Kirk et al., 2014; Marzolini et al., 2014; Prior et al., 2017; Tang et al., 2009), however, to the best of our knowledge, there are no studies regarding carotid shear stress during different types of exercises. Endothelial function relies on shear stress (SS) which is frictional force produced by blood flow and endothelial cells, which can help to determine the efficiency of blood flow.

The purpose of this study was to determine the differences in blood flow patterns across exercise modalities at three different intensities. Therefore, it was hypothesized that greater ESS and Re of the Carotid artery would increase linearly as intensity increases regardless of the exercise modality. In addition, it was also hypothesized that the treadmill at a high intensity would result in greater ESS and Re of the carotid artery compared to the other exercise modalities.

# Methods

## Experimental Design

20 participants were recruited for a repeated measures study design. 6 participants were unable to continue due to the COVID-19 lockdown. Hence, only 14 participants were able to complete the study. The study involved 4 sessions for maximal testing, and 2 sessions of ultrasonography testing. A priori power analysis was conducted in Rstudio using R statistical programing language and the “pwr” library; a total of 14 subjects with stratification by sex (7 per group) at an alpha level (α) of 0.05 with an large effect size (f) of 0.4, was determined to be enough to obtain a power (β) of 0.94 All studies protocols were in accordance the Declaration of Helsinki and were approved by the Institution Review Board at the University of Texas at El Paso (Reference number: 1250657-5).

*Study Protocol*

On the initial visit, participants signed an informed consent form. Following, participants completed a demographics and initial screening questionnaires to determine eligibility. Height and Mass were taken using a calibrated scale and a detecto stadiometer. Thereafter, blood pressure was obtained, and for every visit, hematocrit and resting lactate levels were obtained from the lower end of the earlobe. Then, for visit 1, subjects completed 3 maximal strength tests (Squat, Bench Press, and Biceps curls), then subjects rested for approximately 30 minutes and performed a VO2max treadmill test. On visit 2, participants performed a VO2max Bike followed by 30 minutes rest to perform a VO2max on the Arm Crank.

Maximal oxygen consumption (VO2max) tests were conducted in Bike, Arm Crank, and Treadmill conditions using a metabolic cart (Parvo Medics, TrueOne 2400). A graded exercise test protocol was utilized with speed increased every 2-minutes (Beltz et al., 2016). At 30 seconds before the end of each stage, lactate was drawn from the participants, heart rate was recoded, and rate of perceived exertion was reported. A successful trial was considered if the following criteria were met: 1) Lactate > 4.0 mmol/L, respiratory exchange ratio (RER) > 1.10, heart rate was within 10 bmp of estimated maximal heart rate (220 – age), and RPE < 17 (Beltz et al., 2016). For the subsequent visits, intensities for the VO2 max modalities (Treadmill, bike, and ArmCrank) were be selected as: low = 0-1 mmol/L, moderate = 2-4 mmol/L, and high > 4.0 mmol/L (Rascon et al., 2020).

The 1-RM testing consisted with a familiarization and technique inspection of the individuals exercise execution. Thereafter, participants were asked to predict the maximal load they could achieve. Then, participants performed 5-10 repetitions of the predicted load at a comfortable pace. The load was increased by 20% for the following set and performed for 2-3 repetitions. Then load was increased 5-10lbs until participants reached failure (Montalvo et al., 2021; Seo et al., 2012). Technical execution as well as spotting was performed by a Certified Strength and Conditioning Specialist (CSCS). For the subsequent testing sessions of blood flow patterns, resistance exercise intensities were selected as: low = 45% of 1RM, moderate = 65% 1RM, high = 85% 1RM and the intensities.

*Blood flow pattern testing*

Ultrasound testing (Visit 3 and 4) happened within 24 to 48 hours of visit 2. It will start with a 5-minute warm up, carotid longitudinal imaging were conducted with a cervical probe holder designed by the WM Keck Center for 3D Innovation at UTEP was placed on the subject’s neck, and images were recorded with a Doppler Ultrasound, Linear array, 12 MHz transducer (LA435, Esaote, (MyLab30 Gold, Esaote North America, Inc. in Fishers, IN) (Alvaro N. Gurovich et al., 2021) during an strength test (3 repetitions at 45%1-RM, 65%1-RM, and 85%1-RM of squats, biceps curl, and bench press) and cycling exercise test (2-minutes workload steady-state exercise at low intensity = <2 mmol/L, moderate intensity = 2-4 mmol/L, and high intensity = >4 mmol/L) (Figure 1). Ultrasound images and Doppler signal were analyzed with edge detection technology (Vascular Analysis Integrative System, Medical Imaging Applications, Coralville, IA) and data acquisition system (MP150WSW, BIOPAC Systems Inc., Goleta, CA). Endothelial Shear Stress for antegrade (ESS) and retrograde flow (ESS retrograde) were obtained by the Womersley’s approximation and blood flow patterns via the Reynold’s number which were obtained on mathematical calculations previously described (Dormandy, 1981; Gurovich & Braith, 2012; A. N. Gurovich et al., 2021; Morales-Acuna et al., 2019; Parker et al., 2009; Râdegran, 1997; Rådegran & Saltin, 1998; Shahcheraghi et al., 2002; Simon et al., 1990). Blood flow patterns (Re) were considered undisturbed laminar flow if values were <200, disrober laminar flow if values were between 200-1800, and turbulent flow if values were >2000 (Davies, 2009).

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*INSERT FIGURE 1 ABOUT HERE\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

## Statistical analysis

Data was compiled into a master data spreadsheet (Excel, Microsoft 2021). Data was then exported into Rstudio Integrative Development Environment and analyzed using a custom-built script in R statistical programming language. The “dplyr” package was used for grammar data manipulation, “psych” for data descriptives and reliability, “lm4” and “lmerTest” for linear mixed effects models, for post hoc pairwise comparisons, and for effect sizes.

Reliability of at rest ESS and Re were analyzed using a two-way mixed-factor intra-class correlation coefficient (ICC2,k); “excellent” reliability was considered if > 0.90. Differences between exercise modalities and intensities was assessed using a general linear mixed effects models with adjusting for individual differences as a random effect; model was as follows: dependent variable ~ exercise modality + exercise intensity + Sex + exercise modality\*exercise intensity + (1|Participant). Pairwise differences were analyzed post-hoc with a Holm-Bonferroni p-value correction (*p.adj*) when appropriate; the magnitude of the differences was obtained through a standardized mean difference using Cohen’s D and a Hedge’s g (ESg) correction for small sample size. Statistical significance was set priori at an alpha level of 0.05.

# Results

There was a main effect of intensity, exercise modality, and intensity \* exercise modality on ESS, ESS retrograde, and RE by intensity and condition (p<0.001), but not by Sex (p>0.05). There was significant an interaction of random effect (participant) (p<0.001). Pairwise comparisons during low intensity exercise indicated a difference and large effect between Bike vs Bench, and Squat vs Bench for ESS, and Bike vs ArmCrank on Re (p.adjusted <0.05).Similarly, there was a large effect and difference at moderate exercise intensity between Treadmill vs ArmCrank, Treadmill and Squat, Treadmill vs Bench, Treadmill vs Biceps, and Squat vs Biceps for ESS, and Bike vs ArmCrank, and Squat vs Biceps for Re (p.adjusted <0.05). Finally, there was a difference with a large effect for Bike vs Squat, Bike vs Bench, Bike vs Biceps, ArmCrank and Treadmill, Treadmill vs Squat, Treadmill vs Bench, vs Treadmill vs Biceps for ESS, and Treadmill vs Bench for Re (p.adjusted <0.05) (Table 2 & Figures 2-3).

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*INSERT TABLE 2 and FIGURES 2 and 3 ABOUT HERE \*\*\*\*\*\*\*\*\*\*\*\*\*

For ESS retrograde, there were pairwise difference with a large effect size between Bike vs Biceps, Treadmill vs Squat, Treadmill vs Bench, and Treadmill vs Biceps (p.adjusted <0.05) at a moderate exercise intensity. Additionally, there were pairwise differences with large effect at high exercise intensity between Bike vs ArmCrank, Bike vs Bench, Bike vs Biceps, Treadmill vs Squat, Treadmill vs Bench, and Treadmill vs Biceps (p.adjusted <0.05) (Table 2 & Figure 4).

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# Discussion

The purpose of this study was to determine the effects of different exercise modalities at three different exercise intensities on endothelial shear stress (ESS) and blood flow patterns (Reynold’s number (Re)) at the carotid artery. Our primary hypothesis indicated that running in a treadmill at high intensity produces greater ESS and turbulent blood flow (Re) than any of the other exercise modalities and intensities.

*Limitations*

Our study was limited to the sample size. Our between subject’s comparison could have been compromised to the low sample size (males=7, females=7). However, our results are consistent with previous investigations in our laboratory and we have not found difference in hemodynamic responses to exercises by sex (A. N. Gurovich et al., 2021). Furthermore, our overall sample size was 14 participants, however, this was enough to show differences in responses through the standardized mean difference as denoted by the effect size. Moreover, each of the pairwise comparisons (exercise modality by intensity) yielded a possible 42 comparisons. Thus, in order to avoid the increased chance of committing a type 1 (false positive) and type 2 (false negative) errors we utilized a Holm-Bonferroni correction; the correction utilized presents a more conservative alternative to the fisher’s LSD correction which has been show to produce type 1 error and more liberal to the Bonferroni correction which has also been shown to produce type 2 error. Finally, the results presented in this investigation can only be extrapolated to a similar population (health young male and female participants), and the effects of different exercise modalities and intensities on ESS and Re for clinical populations (i.e. heart conditions) remains unknown.

# Conclusion

Endothelial shear stress is dependant of exercise intensity regardless of the exercise modality. Moreover, running at a high intensity yields the greatest shear stress. Thus, clinicians must take into consideration the hemodynamics at the carotid artery during the different exercise intensities and modalities.

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Figure 1. Typical ultrasound testing setup with neck probe holder utilized during all exercise testing.

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Figure 2. Boxplot of Endothelial Shear Stress (ESS) by exercise modality and intensity.

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Figure 3. Boxplot of Reynolds number (Re) by exercise modality and intensity.

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Figure 4. Boxplot of Endothelial Shear Stress for Retrograde Flow.

Table 1. Descriptives for all Baseline values by group

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | All | | | Males | | | Females | | |
|  | mean | sd | se | mean | sd | se | mean | sd | se |
| Age (yrs.) | 23.00 | 2.86 | 0.76 | 24.00 | 3.56 | 1.35 | 22.00 | 1.63 | 0.62 |
| Height (m) | 1.66 | 0.09 | 0.03 | 1.73 | 0.05 | 0.02 | 1.60 | 0.08 | 0.03 |
| Weight (kg) | 69.18 | 11.03 | 2.95 | 73.94 | 7.60 | 2.87 | 64.41 | 12.37 | 4.68 |
| BMI (kg/m2) | 24.97 | 3.45 | 0.92 | 24.73 | 2.19 | 0.83 | 25.21 | 4.57 | 1.73 |
| SBP | 113.29 | 8.91 | 2.38 | 117.00 | 9.07 | 3.43 | 109.57 | 7.59 | 2.87 |
| DBP | 74.07 | 6.83 | 1.83 | 75.14 | 7.49 | 2.83 | 73 | 6.51 | 2.46 |
| Treadmill VO2(ml/kg/min) | 43.26 | 9.99 | 2.67 | 50.6 | 5.21 | 1.97 | 35.91 | 7.95 | 3.00 |
| Bike VO2(ml/kg/min) | 32.00 | 9.18 | 2.45 | 34.59 | 10.16 | 3.84 | 29.41 | 7.99 | 3.02 |
| ArmCrank VO2(ml/kg/min) | 28.74 | 9.47 | 2.53 | 32.34 | 10.43 | 3.94 | 25.13 | 7.43 | 2.81 |
| 1RM-Squat (kg) | 83.34 | 36.84 | 9.85 | 101.83 | 43.04 | 16.27 | 64.86 | 17.08 | 6.46 |
| 1RM-Bench (kg) | 55.78 | 24.97 | 6.67 | 76.86 | 16.18 | 6.12 | 34.70 | 7.26 | 2.75 |
| 1RM- Biceps (kg) | 33.73 | 20.28 | 5.42 | 47.02 | 21.36 | 8.07 | 20.43 | 4.73 | 1.79 |

Table 2. Endothelial Shear Stress (ESS) and Reynolds number (RE) values by exercise intensity

|  |  |  |  |
| --- | --- | --- | --- |
|  | mean | sd | se |
| *Rest* |  |  |  |
| ESS (dynes/cm2) | 25.14 | 4.28 | 0.81 |
| ESS retrograde (dynes/cm2) | 7.32 | 0.87 | 0.16 |
| Re | 1440.58 | 329.53 | 62.28 |
| *Low intensity* |  |  |  |
| ESS (dynes/cm2) | 41.86 | 13.22 | 1.44 |
| ESS retrograde (dynes/cm2) | 9.79 | 3.76 | 0.41 |
| Re | 2458.73 | 716.33 | 78.16 |
| *Moderate Intensity* |  |  |  |
| ESS (dynes/cm2) | 51.65 | 17.92 | 1.96 |
| ESS retrograde (dynes/cm2) | 11.69 | 6.15 | 0.67 |
| Re | 3014.55 | 918.81 | 100.25 |
| *High Intensity* |  |  |  |
| ESS (dynes/cm2) | 62.19 | 20.99 | 2.29 |
| ESS retrograde (dynes/cm2) | 14.33 | 8.69 | 0.95 |
| Re | 3588.91 | 1128.52 | 123.13 |

Table 3. Pairwise comparisons by exercise intensity for Endothelial Shear Stress (ESS) and Reynolds number (Re).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Intensity | Modality 1 | Modality 2 | t | p.adj | Hedges g | Effect |
| *Endothelial Shear Stress Antegrade Flow (ESS)* | | | | | | |
| Low | Bike | Bench | 3.639 | 0.042 | 0.915 | Large |
| Low | Squat | Bench | 3.950 | 0.025 | 0.994 | Large |
| Moderate | Bike | Bench | 3.739 | 0.022 | 0.940 | Large |
| Moderate | Bike | Biceps | 3.847 | 0.022 | 0.968 | Large |
| Moderate | ArmCrank | Treadmill | -4.081 | 0.017 | -1.027 | Large |
| Moderate | Treadmill | Squat | 3.965 | 0.019 | 0.997 | Large |
| Moderate | Treadmill | Bench | 6.723 | 0.000 | 1.691 | Large |
| Moderate | Treadmill | Biceps | 5.742 | 0.001 | 1.444 | Large |
| Moderate | Squat | Biceps | 3.811 | 0.022 | 0.959 | Large |
| High | Bike | Squat | 3.382 | 0.044 | 0.851 | Large |
| High | Bike | Bench | 5.218 | 0.002 | 1.312 | Large |
| High | Bike | Biceps | 4.548 | 0.005 | 1.144 | Large |
| High | ArmCrank | Treadmill | -5.283 | 0.002 | -1.329 | Large |
| High | Treadmill | Squat | 7.487 | 0.000 | 1.883 | Large |
| High | Treadmill | Bench | 7.289 | 0.000 | 1.834 | Large |
| High | Treadmill | Biceps | 7.725 | 0.000 | 1.943 | Large |
| *Endothelial Shear Stress Retrograde Flow (ESSretrograde)* | | | | | | |
| Moderate | Bike | Biceps | 3.824 | 0.030 | 0.962 | large |
| Moderate | Treadmill | Squat | 3.728 | 0.033 | 0.938 | large |
| Moderate | Treadmill | Bench | 3.724 | 0.033 | 0.937 | large |
| Moderate | Treadmill | Biceps | 3.847 | 0.030 | 0.968 | large |
| High | Bike | ArmCrank | 4.489 | 0.009 | 1.129 | large |
| High | Bike | Bench | 3.667 | 0.037 | 0.922 | large |
| High | Bike | Biceps | 3.573 | 0.038 | 0.899 | large |
| High | Treadmill | Squat | 3.632 | 0.037 | 0.914 | large |
| High | Treadmill | Bench | 4.089 | 0.018 | 1.029 | large |
| High | Treadmill | Biceps | 3.520 | 0.038 | 0.886 | large |
| *Reynolds Number (Re)* | | | | | | |
| Low | Bike | ArmCrank | 4.227 | 0.015 | 1.063 | Large |
| Moderate | Bike | ArmCrank | 3.723 | 0.036 | 0.936 | Moderate |
| Moderate | Squat | Biceps | 3.799 | 0.033 | 0.956 | Moderate |
| High | Treadmill | Bench | 3.865 | 0.029 | 0.972 | High |