2019-07-01

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The Relationship Between Vascular Endothelial Function and Peak Exercise Blood Flow

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A thesis submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Master of Science

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ABSTRACT

The Relationship Between Vascular Endothelial Function and Peak Exercise Blood Flow

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Purpose The vascular endothelium is an influential contributor to vasodilation at rest, yet its role during peak exercise is relatively unknown. The purpose of this study is to determine if exercise leg blood flow during dynamic submaximal and maximal exercise is related to resting vascular endothelial function. **Methods** Nineteen subjects (aged 23 ± 0.57 yr) completed multiple assessments of vascular endothelial function including passive leg movement (PLM), rapid onset vasodilation, (ROV) and flow-mediated dilation (FMD). Peak muscle blood flow was assessed during single leg knee extension (KE) exercise. Doppler ultrasound of the femoral artery was utilized to assess muscle blood flow. **Results** Peak exercise blood flow was linearly related with microvascular endothelial function determined by PLM ($P < 0.001$) and ROV ($P < 0.001$). Normalizing muscle blood flow for quadriceps mass did not change this significant association. Individuals with high vascular endothelial function had greater muscle blood flow during KE compared to those with low endothelial function ($P = 0.05$). Post hoc analysis indicated a significant difference in blood flow between high and low endothelial function groups at 20 W, 30 W, and peak flow ($P = 0.042, 0.048, 0.001$, respectively). **Conclusion** Peak muscle blood flow during dynamic exercise is correlated with vascular endothelial function, as measured by PLM and ROV, accounting for between 30 to 50% of the variance in this relationship. These data support the hypothesis that endothelial function significantly contributes to the peak blood flow response during dynamic exercise.

Keywords: endothelial function, blood flow, passive leg movement, rapid onset vasodilation, flow-mediated dilation

ACKNOWLEDGEMENTS

To the many people who have helped and supported me in my experience in this program: First and foremost, to my wife Stephanie, for her support and amazing love as a wife and mother. To my amazing committee members, Dr. Pat Vehrs and Dr. Gary Mack, for their dedicated work, and prompt response and input. To my chair and advisor, Dr. Jayson Gifford, for his willingness to teach and guide me, for his patience, not giving up on me, and for all those times he answered his office door when I came knocking. I'm glad he did! To all the research assistants who helped with data collection, especially to Meagan Proffit for all her hard work. To my fellow graduate students for helping me get through all the hard times. And, lastly, to the department of Exercise Sciences and Brigham Young University for the opportunity to achieve this great honor.

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INTRODUCTION

The ability of the body to increase blood flow to skeletal muscle in response to a stimulus influences one's ability to perform and sustain exercise (1). While rates of muscle blood flow are heavily influenced by the rate of work being performed, there is considerable subject-to-subject variability in the rate of muscle blood flow observed at a given work rate. For example, Garten et al. (2) observed a range of quadriceps muscle blood flow of about 1,500 $ml \cdot min^{-1}$ when healthy young males performed 25 W of single leg knee extension exercise (KE). Moreover, peak blood flow also varies with fitness level. Blood flow in endurance-trained individuals, for example, has been shown to reach 1,000–2,000 $ml \cdot min^{-1}$ greater than age-matched untrained individuals (3).

The flow of fluid (blood), as described by Poiseuille's law $\dot{Q} = (\Delta P \pi r^4)/8Ln$, is heavily influenced by change in blood pressure across the muscle (ΔP) and the radius (r) of vascular network (i.e., cross-sectional area of vascular network). The ability of the muscle to increase blood flow during exercise is influenced by multiple factors including vascular density, limb size, sympathetic tone, and vasodilatory capabilities which would affect the total change in radius or cross–sectional area in the vascular bed of the exercising muscle (4–6). As radius is raised to the fourth power, the vasodilatory capacity is of great importance in achievement of higher blood flow.

One of the most influential contributors to vasodilation is the vascular endothelium, which releases vasodilator substances, such as nitric oxide (NO), prostaglandins, and hyperpolarizing factors (7–10). Enhancements to the endothelium's ability to produce these substances increase the vasodilatory capacity, leading to increased blood flow to meet O_2 demand (11,12). Given the importance of muscle blood flow, it seems probable that enhanced endothelial function may contribute to greater peak blood flow and O_2 delivery during exercise (3).

Vascular endothelial function is clearly a major contributor to the regulation of blood flow at rest, but its role during exercise remains unclear (13,14). For example, Frandsen et al. (15) observed no change in blood flow during KE exercise after inhibiting NO synthase, indicating that NO is not essential for exercise blood flow. Nevertheless, they did observe a ~20% reduction in vascular conductance (expressed as blood flow/mean arterial pressure, i.e., an index of vasodilation) during KE when NO synthase was inhibited. From subsequent studies which simultaneously inhibited multiple endothelium-derived vasodilators, a redundant mechanism theory has arisen, indicating that additional vasodilatory mechanisms may compensate when the function of another endothelium-dependent dilator is attenuated (8,16). For example, Mortensen et al. (8) demonstrated that blood flow during light intensity KE was unchanged after the prostaglandins dilator system was inhibited, but subsequently decreased when NO and prostaglandins production were inhibited simultaneously. In contrast, animal studies have found that blood flow and vascular conductance are attenuated during exercise with inhibition of NO (17,18).

Many different methods have been used to assess vascular endothelial function. The flow–mediated dilation (FMD) assessment of endothelial function, based on blood flow regulation in conduit vessels, has become common place in laboratories (19,20). The more simple and practical methods of passive leg movement (PLM) (21) and rapid onset vasodilation (ROV) (22,23), both of which are dependent on NO bioavailability (14,24), are emerging as insightful endothelial function assessments. The PLM and ROV assessments both measure the initial hemodynamic response to exercise. Based primarily on the dilation of microvascular rather than the conduit arteries (14,21), PLM and ROV may provide a unique understanding of what drives the increases in leg blood flow during exercise.

While vascular endothelial function has been shown to be greater in individuals who are active (25), the role that vascular endothelial function plays in exercise leg blood flow, including peak exercise blood flow, has not thoroughly been documented. With the endothelium playing an important role in vasodilation, the purpose of this study is to determine if leg blood flow during maximal and submaximal KE exercise in young healthy adults is related to vascular endothelial function assessed by FMD, PLM, and ROV at rest.

METHODS

Subjects

Twenty–one young, healthy subjects were recruited for this study, of which two subjects were disqualified due to the inability to perform knee extension exercise. Thus, 19 subjects (12 male, 7 female, 18–30 years old) completed the present study. All subjects were healthy, nonobese, nonsmokers, and free from medications that would affect their hemodynamic responses to exercise (21). Females participating in the study had all data collected within the first seven days of the menstrual cycle (26,27). Subjects completed a prescreening questionnaire and reviewed the informed consent form with the primary investigator prior to providing written and informed consent. This study was approved by the Institutional Review Board (IRB) at Brigham Young University (BYU), and performed in accordance with the *Declaration of Helsinki*.

Procedures

Subjects reported to the laboratory on two occasions, well rested, having fasted for 4 hours, and having refrained from exercise and consumption of alcohol or caffeine for ~24 hours

(19,21). Each visit was separated by a minimum of 24 hours. All data collection and exercise were completed on the subject's right leg, regardless of leg dominance.

Prior to the main experiment, body measurements including height (cm) and weight (kg) were measured, and body mass index (BMI, $kg \cdot m^{-2}$) was calculated. Additionally, quadriceps muscle volume was measured noninvasively, as described by Layec et al. (28), with a combination of limb circumference measurements and skin fold thickness measurements. This method strongly correlated to measures of thigh and quadriceps muscle volume assessed by MRI. Muscle volume measurements were then converted to quadriceps muscle mass by assuming a muscle density of $1.49\ kg \cdot L^{-1}$ (29).

Assessment of Single Leg Maximum Voluntary Contraction

Maximum voluntary contraction (MVC) for weighted knee extension on the right leg was completed on a cable weighted knee extension machine. Subjects completed a warm-up set of 10 repetitions at 40–60% of estimated MVC. Following a period of 2 min rest, 5 repetitions at 60–80% of estimated MVC was completed. Performance of 3–4 more attempts with increasing weight were completed until an MVC was achieved. The greatest amount of weight the subject was able to lift with full knee extension was recorded as the MVC.

Assessment of Endothelial Function

Passive Leg Movement (PLM): Endothelial function was assessed via PLM, according to recently published procedures (11,21). Specifically, subjects were seated in an upright position with knees fully extended (180°). Researchers controlled the movement of the leg back and forth from the extended position of the knee (180°) to the flexed position (90°), at a rate of 60 knee extensions per min, while the subjects stayed relaxed with no voluntary muscle activation (30). Cadence of the knee flexion and extension was established using a metronome. A knee brace set to allow 90° of knee flexion/extension was worn throughout the PLM assessment. This procedure was completed three times with a 10–15-min period of rest between each attempt. Femoral artery blood flow and mean arterial pressure (MAP) were measured throughout the assessment utilizing Doppler ultrasound and finger photoplethysmography, respectively. Blood flow data were subsequently analyzed second-by-second and a 3-s rolling average was applied to smooth the data. The peak blood flow and the area under the curve (total blood flow) were identified for each of the three 1-min trials and then averaged together (21). The data presented in this manuscript are the average of the three trials. A more detailed description of the ultrasound measurements is provided below.

Rapid Onset Vasodilation (ROV): After completion of the PLM assessment, subjects rested 10–15 min before completing the ROV assessments of vascular endothelial function (22). Subjects were seated in an upright position with legs hanging over the end of the seat of a cable-weighted machine with knees in a flexed position. Following 1 min of baseline data collections, subjects extended the knee to ~180° and then back to 90° with no engagement of knee flexor or extensor muscles during the knee flexion phase (e.g., full extension and then relax). All subjects completed two trials at an absolute work set at 60 Newton meters (Nm), each separated by 2-min rest (22,31). As work is the product of mass, displacement distance, and gravity, the mass each subject lifted was determined based upon their displacement distance, measured during the MVC protocol, to ensure the performance of 60 Nm during each extension. Femoral blood flow and MAP were gathered for 1-min baseline prior to contraction, immediately followed by collection during contraction and 1-min recovery utilizing Doppler ultrasound and finger photoplethysmography. Data were subsequently analyzed second-by-second and a 3-s rolling average was applied to smooth the data. The peak blood flow and the area under the curve were identified for each 1-min trial and then averaged together.

Flow Mediated Dilation (FMD): On a different day, separated by at least 24 hours, subjects reported to the lab well rested and in a fasted state (~4 hours) to have endothelial function assessed via FMD. Procedures for FMD were performed in accordance with current recommendations (20) and specifically completed on the superficial femoral artery (32). While lying in the supine position, a 9 cm blood pressure cuff (D. E. Hokanson Inc., Bellevue, WA, USA) was placed on the upper leg just proximal to the knee cap. Baseline measurements (diameter and blood flow) were gathered for 1 min as the ultrasound probe was placed over the superficial femoral artery approximately 10 cm proximal to the cuff. The cuff was then inflated for 5 min at suprasystolic pressures (~250 mmHg). Prior to the release of the cuff, 30 s of blood flow data was collected, immediately followed by 2 min of data collection after the release of the cuff pressure. Diameter of the superficial femoral artery was measured for FMD assessment during the last 30 s of occlusion and 2 min following the release of the cuff pressure. Diameter was analyzed frame-by-frame by automated edge detection software (Quipu srl., Pisa, Italy) and averaged into 1-s bins corresponding with 1-s average velocities. Superficial femoral artery blood velocity was assessed with a Doppler frequency of 5 MHz operated in the high-pulsed repetition frequency mode. A 3-s rolling average was applied to smooth diameter and velocity data. FMD measurements were expressed as a percent change in diameter and calculated with the equation:

$$FMD\ (\%) = \frac{(Peak\ Diameter - Baseline\ Diameter)}{Baseline\ Diameter}\ x\ 100$$

while peak flow following the release of the cuff (i.e., $_{FMD}Flow_{Peak}$) was identified as the greatest 1-s average of flow achieved following cuff release (20).

Knee Extension Assessment of Blood Flow

Knee Extension Familiarization: Following these assessments, subjects were given time to familiarize themselves with dynamic knee extension exercise, which would ultimately be used to determine peak exercise blood flow (i.e., ${}_{KE}Flow_{Peak}$). During this familiarization and subsequent knee extension tests, subjects were seated in an upright position, with the right leg attached to the knee extension ergometer (a modified Monarch cycle ergometer), as first described by Andersen et al. in 1985 (33). During this exercise subjects were instructed to participate only in active knee extension exercise, with no active knee flexion, thus isolating the majority of the work to the quadriceps femoris of the thigh. With visible feedback of cadence, subjects were instructed to extend the knee at a rate of 60 rpm for the duration of the exercise.

Knee Extension Maximum Work Rate Assessment: After familiarization, a graded exercise test to determine maximum knee extension work rate (WR_{max}) for dynamic single leg knee extension on the right leg was completed. Starting at 10 W, subjects began extending the leg at a rate of 60 rpm for a warm-up of 3 min. Following this warm-up, work rate increased by 5–10 W (depending upon subjects' perceived effort) every minute thereafter until the subject could no longer maintain a frequency of 60 rpm despite verbal encouragement. The highest power output completed for 1 min was identified as WR_{max}.

Femoral Artery Blood Flow During Dynamic Knee Extension: On a separate day, while well rested, subjects completed 3-min bouts of six exercise intensities of single leg KE exercise (10 W, 20 W, 30 W, and 75%, 90% and 100% predetermined WR_{max}). If subjects were able to complete 100% WR_{max}, following ~5 min of rest, work rate (WR) was increased to 110% of the predetermined WR_{max} and so on until failure. The first three work rates (10–30 W) were completed sequentially, totaling 9 min of exercise. A period of ~5 min of active recovery, at a low intensity and rpm, followed the exercise. The last three work rates (75–100% WR_{max}) were each completed with ~5 min of active recovery between each bout. All data, including blood flow and MAP for all work rates, were recorded during the final 1 min of each 3-min period, as described in *Doppler Ultrasound* and *Finger Photoplethysmography*, respectively.

Blood Flow and Vascular Conductance Measurements

Doppler Ultrasound: Femoral artery blood flow was measured second-by-second for 60 s for PLM, ROV, and FMD assessments, and 12-s averages were measured during KE for the last 1 min of each workload. Leg blood flow during PLM, ROV, and KE was assessed utilizing Doppler ultrasound of the common femoral artery, proximal to the exercising muscles. Specifically, measurements of common femoral artery blood velocity and artery diameter, 2–3 cm proximal to the superficial/deep bifurcation, were taken using a Logiq E ultrasound Doppler system in duplex mode (General Electric Medical Systems, Milwaukee, WI, USA) equipped with a linear array transducer function at a B-mode frequency of 9 MHz and a Doppler frequency of 5 MHz. Blood velocity was assessed with an insonation angle of no more than 60°, while the sample size was maximized and centered according to vessel size and position in real time. Ultimately, femoral blood flow ($ml \cdot min^{-1}$) was calculated using the equation:

$$\text{Femoral blood flow} = [(\text{mean blood velocity}) \times (\pi \times (\text{vessel radius}^2)) \text{ x } 60\text{s}]$$

Where mean blood velocity is expressed in $cm \cdot s^{-1}$ and radius is expressed in cm.

Finger Photoplethysmography: Blood pressure was measured continuously with a finger photoplethysmography system (CNAP, CNSystems, Graz, Austria) using the vascular unloading technique of Peňáz (34). MAP was calculated as the pressure-time integral of the continuous finger blood pressure measurement (21).

Statistical Analysis

All data are expressed as the mean ± SE. Repeated-measures ANOVA, followed by a Tukey's post hoc test, was used to identify the effect of KE WR on blood flow. Correlations between relevant variables were evaluated using Pearson correlation coefficients, with alpha set at $P < 0.05$ *a priori*. A stepwise linear regression for $_{KE}Flow_{Peak}$ was completed using $_{PLM}Flow_{Peak}$, $_{ROV}Flow_{Peak}$, FMD, quadriceps mass, and KE WR_{max} as independent variables. Repeated measures analysis of variance (ANOVA) was used to compare blood flow across KE WR. A mixed-model ANOVA with repeated measures for work rate and two independent groups was used to test differences in $_{KE}Flow_{Peak}$ between individuals with high and low endothelial function. When significance was detected, Tukey's post hoc identified between-group differences. All statistical analyses were completed using SPSS version 21 (SPSS Inc., Chicago, IL, USA).

RESULTS

Nineteen young (age 23 ± 0.57 yr), healthy individuals (12 male, 7 female) completed the present study. See Table 1 for subject characteristics.

Blood Flow Response to Knee Extension Exercise

As shown in Figure 1, there was a main effect of work rate on blood flow during knee extension exercise (KE) within subjects. Blood flow increased with each increase in WR ($P < 0.001$) until WR_{max}, which did not significantly differ from 90% WR_{max} to 100% WR_{max} ($P = 0.17$). Peak flow was identified as the highest blood flow achieved, regardless of WR. Blood flow increases in a linear manner from rest (380.1 ± 27.51 $ml \cdot min^{-1}$) to peak exercise (52.6 ± 4.08 W, 4310.47 ± 217.88 $ml \cdot min^{-1}$) (Table 1).

Relationship Between Peak Exercise Blood Flow and Vascular Endothelial Function

As illustrated in Figure 2, among the three assessments of vascular endothelial function there was a significant positive, linear correlation between peak blood flow elicited from KE (${}_{\mathrm{KE}}\mathrm{Flow}_{\mathrm{Peak}}$) and peak blood flow from both PLM (${}_{\mathrm{PLM}}\mathrm{Flow}_{\mathrm{Peak}}$) (r = 0.77, *P* = 0.001; Figure 2A) and ROV (${}_{\mathrm{ROV}}\mathrm{Flow}_{\mathrm{Peak}}$) (r = 0.69, *P* = 0.001; Figure 2B). The ${}_{\mathrm{PLM}}\mathrm{Flow}_{\mathrm{Peak}}$ and ${}_{\mathrm{ROV}}\mathrm{Flow}_{\mathrm{Peak}}$ were 1434.6 ± 102.23 ml·min^{-1} and 1951.45 ± 132.1 ml·min^{-1}, respectively (Table 1). FMD had no significant correlation with ${}_{\mathrm{KE}}\mathrm{Flow}_{\mathrm{Peak}}$ (r = -0.02, *P* = 0.86; Figure 2C), with an average percent dilation increase across all subjects of 6.15 ± 0.64 % (Table 1). Although no significant correlation was found between endothelial function and blood flow at 10 W, there was significance at 20 W with ${}_{\mathrm{ROV}}\mathrm{Flow}_{\mathrm{Peak}}$ (*P* = 0.04) and ${}_{\mathrm{PLM}}\mathrm{Flow}_{\mathrm{Peak}}$ trending towards significance (*P* = 0.06).

When normalizing for quadriceps mass, the correlation between ${}_{\mathrm{PLM}}\mathrm{Flow}_{\mathrm{Peak}}$ and ${}_{\mathrm{KE}}\mathrm{Flow}_{\mathrm{Peak}}$ remained significant (r = 0.62, *P* = 0.001; Figure 3A), as well as the correlation between ${}_{\mathrm{ROV}}\mathrm{Flow}_{\mathrm{Peak}}$ and ${}_{\mathrm{KE}}\mathrm{Flow}_{\mathrm{Peak}}$ (r = 0.51, *P* = 0.05; Figure 3B). When normalized for quadriceps mass, FMD exhibited a significant positive, linear correlation with ${}_{\mathrm{KE}}\mathrm{Flow}_{\mathrm{Peak}}$ (r = 0.45, *P* = 0.05; Figure 3C). Additional correlations of interest are listed in Tables 2 and 3.

At an absolute work rate of 30 W, there was a significant positive, linear correlation between femoral artery blood flow at 30 W (${}_{\mathrm{KE}}\mathrm{Flow}_{30}$) and ${}_{\mathrm{PLM}}\mathrm{Flow}_{\mathrm{Peak}}$ (r = 0.47, *P* = 0.04), and ${}_{\mathrm{ROV}}\mathrm{Flow}_{\mathrm{Peak}}$ (r = 0.67, *P* = 0.03) (Table 2). FMD showed no significant correlation with ${}_{\mathrm{KE}}\mathrm{Flow}_{30}$ (r = -0.12, *P* = 0.62). Normalizing for quadriceps mass (Table 3), the correlation between ${}_{\mathrm{PLM}}\mathrm{Flow}_{\mathrm{Peak}}$ and ${}_{\mathrm{KE}}\mathrm{Flow}_{30}$ was no longer significant (r = 0.32, *P* = 0.18), likewise, the correlation between ${}_{\mathrm{ROV}}\mathrm{Flow}_{\mathrm{Peak}}$ and ${}_{\mathrm{KE}}\mathrm{Flow}_{30}$ was weakened (r = 0.38, *P* = 0.11). In contrast, FMD exhibited a significant positive, linear correlation with ${}_{\mathrm{KE}}\mathrm{Flow}_{30}$ (r = 0.58, *P* = 0.01). No significant correlation was found between endothelial function and blood flow at 10 W (all $P >$ 0.05). At 20 W there was a significant relationship between ${}_{\text{ROV}}\text{Flow}_{\text{Peak}}$ (r = 0.47, P = 0.04) and ${}_{\text{KE}}\text{Flow}$, and a tendency for a relationship between and ${}_{\text{PLM}}\text{Flow}_{\text{Peak}}$ and ${}_{\text{KE}}\text{Flow}$ (r = 0.43, P = 0.07).

Relationship Between Peak Vascular Conductance and Vascular Endothelial Function

Among the three assessments of vascular function, there was a significant positive, linear correlation between peak vascular conductance elicited from KE exercise (${}_{\text{KE}}\text{Conductance}_{\text{Peak}}$) and peak vascular conductance from PLM (${}_{\text{PLM}}\text{Conductance}_{\text{Peak}}$) (r = 0.51, P = 0.04) (Table 2). No correlation was found with ROV (${}_{\text{ROV}}\text{Conductance}_{\text{Peak}}$) (r = -0.15, P = 0.55). FMD also had no significant association with ${}_{\text{KE}}\text{Conductance}_{\text{Peak}}$ (r = -0.08, P = 0.74; Figure 2C). All other important correlations are listed in Table 2.

Correlation Between Blood Flow During KE and Vascular Endothelial Function when Controlling for Quadriceps Mass and Power Output

Partial correlations, controlling for quadriceps mass and power output during KE exercise, were performed to determine if the relationship between endothelial function and KE blood flow exists independently of these factors. Controlling for quadriceps mass, ${}_{\text{KE}}\text{Flow}_{\text{Peak}}$ was found to still have a significant correlation with ${}_{\text{PLM}}\text{Flow}_{\text{Peak}}$ (r = 0.64, P = 0.004), ${}_{\text{PLM}}\text{Flow}_{\text{AUC}}$ (r = 0.62, P = 0.007), and ${}_{\text{ROV}}\text{Flow}_{\text{Peak}}$ (r = 0.57, P = 0.01) (Table 4A). Controlling for work rate performed during maximal KE, ${}_{\text{KE}}\text{Flow}_{\text{Peak}}$ was found to still have a significant correlation with ${}_{\text{PLM}}\text{Flow}_{\text{Peak}}$ (r = 0.70, P = 0.001), ${}_{\text{PLM}}\text{Flow}_{\text{AUC}}$ (r = 0.63, P = 0.005), and ${}_{\text{ROV}}\text{Flow}_{\text{Peak}}$ (r = 0.61, P = 0.007) (Table 4B). Additional partial correlations of interest are listed in Tables 4A and 4B.

Stepwise Linear Regression

A stepwise linear regression was completed for the dependent variable $_{KE}Flow_{Peak}$, with specific predictor variables: $_{PLM}Flow_{Peak}$, $_{ROV}Flow_{Peak}$, FMD, quadriceps mass, and KE WR_{max}. The strongest predictor was identified as $_{PLM}Flow_{Peak}$, with the equation $Flow = 1981.6 + 1.639(_{PLM}Flow_{Peak})$; ($r^2 = 0.592$ $P = 0.001$), with flow being expressed in $ml \cdot min^{-1}$. KE WR_{max} was the only other predictor variable that significantly added to the prediction equation ($r^2 = 0.749$, $P < 0.001$), with the final equation being: $Flow = 1367.7 + 1.181(_{PLM}Flow_{Peak}) + 24(KE\ WR_{max})$, with an r^2 change of 0.157 ($P = 0.006$).

Blood Flow During Knee Extension in Individuals with Low and High Endothelial Function

After completing the study, the effect of endothelial function, assessed by $_{PLM}Flow_{Peak}$, on $_{KE}Flow$ was examined by splitting the data into two groups, a low endothelial function group (bottom 8 subjects) and high endothelial function group (top 8 subjects), based upon absolute $_{PLM}Flow_{Peak}$ and expressed in $ml \cdot min^{-1}$. The remaining 3 subjects' data was unused as to create a distinct separation between the two response groups. Table 5A shows the hyperemic response to increasing WR during KE for the two groups. There was a significant main effect of work rate within subjects, such that increasing work rate was associated with increased blood flow ($P < 0.001$). There was also a significant main effect of group such that the high endothelial function group had greater blood flow overall compared to the low endothelial function group ($P = 0.05$). Post hoc analysis indicated a significant difference in blood flow between groups at 20 W, 30 W, and peak flow ($P = 0.042, 0.048, 0.001$, respectively). Overall, the low endothelial function group had smaller quadriceps mass and contained a greater proportion of females to males compared to the high endothelial function group. Subject characteristics for each group are listed in Table 5A.

In Table 5B, subjects were divided into high and low endothelial function groups based upon $_{\mathrm{PLM}}\mathrm{Flow}_{\mathrm{Peak}}$, normalized for quadriceps mass and expressed as $\mathrm{ml \cdot min^{-1} \cdot kg^{-1}}$. Note that these groups are not made up of the same subjects as in Table 5A. When normalizing for quadriceps mass, a significant main effect of endothelial function group was observed ($P = 0.05$), such that those with a higher PLM response achieved significantly higher blood flow per kg of quadriceps mass at any given work rate. Tukey's least significant difference post hoc indicated a significant difference in blood flow per kg of quadriceps mass between groups at 30 W and peak flow ($P = 0.025$, and 0.008, respectively). Subject characteristics for each of these groups are listed in Table 5B.

DISCUSSION

In the present study, the use of multiple assessments of vascular endothelial function (PLM, ROV, FMD) and a valid measurement of peak leg blood flow (single leg knee extension; Figure 1) (35) were used to evaluate the relationship between endothelial function and exercise blood flow. There were two novel findings demonstrated in this study: 1) $_{\mathrm{KE}}\mathrm{Flow}_{\mathrm{Peak}}$ was significantly correlated with assessments of vascular endothelial function, particularly those assessing microvascular function and 2) that individuals with high vascular endothelial function demonstrated a greater $_{\mathrm{KE}}\mathrm{Flow}$ at submaximal and maximal KE work rates. These findings highlight the important contribution of endothelial function to peak muscle blood flow during dynamic KE exercise.

Relationship Between Endothelium-Dependent Dilation and Peak Exercise Flow

Maximum blood flow during exercise is the product of a number of components (4). Though there are a number of factors involved in the control of peak exercise blood flow, this study explored three important factors: vasodilatory capability, muscle mass, and perfusion pressure and their relationship during peak exercise.

Vasodilatory Capability: Microvascular Endothelial Function

The hyperemic response to the PLM is highly related to the function of the vascular endothelium (14,32,36) especially that of the microvasculature (21). Figure 2A shows that greater ${}_{\mathrm{PLM}}\mathrm{Flow}_{\mathrm{Peak}}$ was associated with a higher ${}_{\mathrm{KE}}\mathrm{Flow}_{\mathrm{Peak}}$ ($P < 0.001$). The hyperemic response to PLM is highly dependent on NO bioavailability (~80%) (14,37,38). As such, the close association between ${}_{\mathrm{PLM}}\mathrm{Flow}_{\mathrm{Peak}}$ and ${}_{\mathrm{KE}}\mathrm{Flow}_{\mathrm{Peak}}$ suggests that endothelial function is involved in this relationship. The hemodynamic response to ROV has a similar endothelial component to that of PLM but also includes mechanical and adrenergic factors (23). The ROV assessment is also a good indicator of NO-dependent vasodilation (23,24), and, much like PLM, is reflective of microvascular function. In agreement with the PLM response, Figure 2B shows that greater ${}_{\mathrm{ROV}}\mathrm{Flow}_{\mathrm{Peak}}$ elicited higher ${}_{\mathrm{KE}}\mathrm{Flow}_{\mathrm{Peak}}$ ($P < 0.001$). Mechanical factors such as shear stress and stretch are the likely cause of NO production. As there are higher rates of both shear stress and stretch during peak exercise, it is possible that the endothelial-derived production of vasodilators increases during these times, subsequently leading to greater vasodilation and ${}_{\mathrm{KE}}\mathrm{Flow}_{\mathrm{Peak}}$.

The relationship between microvascular function and exercise blood flow is further supported by the significant relationship between ${}_{\mathrm{KE}}\mathrm{FLOW}_{\mathrm{PEAK}}$ and the reactive hyperemia response to cuff occlusion (${}_{\mathrm{FMD}}\mathrm{FLOW}_{\mathrm{PEAK}}$), which is reflective of the microvascular function in an entirely different vascular bed (calf) than was exercised during knee extension (quadriceps) (39). Thus, the endothelial function of the microvasculature appears to be strongly related to the peak exercise flow.

Vasodilatory Capability: Conduit Artery Endothelial Function

In contrast to the assessment of the microvascular endothelial function with PLM and ROV, the FMD assessment is indicative of conduit artery function (40,41). These measurements in conduit arteries (i.e., brachial or superficial femoral) have long been established as an assessment of endothelial-derived NO bioavailability (20,42). Recent findings suggest, however, that FMD should be viewed more as endothelium-dependent vasodilation and not necessarily NO-dependent vasodilation (43,44), separating itself from PLM and ROV. In the present study, contrary to PLM and ROV, no correlation was found between FMD and $_{KE}Flow_{Peak}$ (Figure 2C). The absence of a correlation may be due to many factors including the effects of muscle mass, artery size, and sympathetic activity on FMD (45). One of these potential reasons is the strong inverse relationship between artery or leg size and FMD (46). Green et al. (47) suggest that individuals with increased lumen size and wall thickness, which may be more common in those with larger legs, tend to present with lower FMD response. The increased size of the artery means there is less need for more dilation in the presence of an ischemic stimulus. It is important to note that in this scenario, while the FMD was assessed on an artery in the thigh (superficial femoral), the artery is not a major supplier of the muscle active during KE (i.e., quadriceps femoris). Thus, the assessment of FMD in this study was not as linked to the quadriceps utilized during KE as PLM and ROV were, which mostly assess the vascular function in the quadriceps. This may also be part of the reason for a lack of relationship between FMD and $_{KE}Flow_{Peak}$.

Relationship Between Muscle Mass and Peak Exercise Flow

The size of the vascular network, comprised of the density of arterioles and capillaries as well as leg muscle size, has a large bearing on the hyperemic response to an exercise stimulus (48). In an attempt to clarify the involvement of the endothelium during exercise, data were analyzed both mathematically, normalizing for quadriceps mass (i.e., dividing by quadriceps mass, Figure 3), and statistically, controlling for quadriceps mass (Table 3 and 4A). As illustrated in Figure 3, the correlation between ${}_{\text{PLM}}\text{Flow}_{\text{Peak}}$ and ${}_{\text{KE}}\text{Flow}_{\text{Peak}}$ remains significant (Figure 3A, $P < 0.001$), and the correlation between ${}_{\text{ROV}}\text{Flow}_{\text{Peak}}$ and ${}_{\text{KE}}\text{Flow}_{\text{Peak}}$ also remains significant (Figure 3B, $P < 0.05$). Notably, when normalizing for quadriceps muscle mass, the relationship between FMD and ${}_{\text{KE}}\text{Flow}_{\text{Peak}}$ becomes significant (Figure 3C). Moreover, when statistically controlling for quadriceps mass (i.e., partial correlation), multiple indices of endothelial function, including ${}_{\text{PLM}}\text{Flow}_{\text{Peak}}$, are still related to ${}_{\text{KE}}\text{Flow}_{\text{Peak}}$ (Table 4A). These findings indicate that ${}_{\text{KE}}\text{Flow}_{\text{Peak}}$ is related to these assessments of vascular endothelial function independent of leg mass, further supporting the relevance of the endothelium in ${}_{\text{KE}}\text{Flow}_{\text{Peak}}$ (2).

An additional component to the vascular network is the actual density of the network, or the number of arterioles or capillaries per unit of volume within the muscle. Indeed, blood flow increases as the number of potential pathways to accept blood increases (i.e., increasing the total cross-sectional area of the vascular network). Exercise training has been shown to greatly increase vascular density even among muscle groups with lower mass (49). While Robbins et al. (48) reported no relationship between capillary density and peak reactive hyperemia (i.e., ${}_{\text{FMD}}\text{Flow}_{\text{Peak}}$), it is possible that part of the relationship between our indices of vascular function and peak exercise flow could be mediated by differences in vascular/capillary density. Without measurements to quantify vascular density in this study, it is not possible to confirm nor rule out the role of vascular density in the relationships observed in this study. As these measurements were beyond the scope of this study, future investigations should seek to include these measures.

Relationship Between Perfusion Pressure and Peak Exercise Flow

As mentioned, in addition to changes in total cross-sectional area of the vascular bed, blood flow to a muscle is also influenced by perfusion pressure. Notably, MAP did not exhibit a significant relationship with ${}_{KE}FLOW_{PEAK}$ (Figure 2D), supporting the notion that differences in blood flow are more related to vasodilatory capacity than to differences in perfusion pressure.

Summary

To determine which of the above factors exhibited a significant independent relationship with ${}_{KE}Flow_{Peak}$, a stepwise linear regression analysis was performed with the various assessments of endothelial function, quadriceps mass, work rate during peak flow, and MAP during peak flow as predictor variables. Notably, endothelial function, assessed as ${}_{PLM}Flow_{Peak}$, and the work rate during peak flow were the only two significant independent predictors of ${}_{KE}Flow_{Peak}$, with ${}_{PLM}Flow_{Peak}$ being the most predictive and significant of the two. Thus, endothelial function, assessed by PLM, is even more predictive of peak flow during exercise than the work rate of the exercise, the size of the active muscle, and the perfusion pressure.

Comparison of High and Low PLM Responders to Blood Flow

To gain greater understanding of the role of the endothelium during exercise blood flow, we compared the hyperemic response to exercise of individuals with low ${}_{PLM}Flow_{Peak}$ response (L-PLM) to individuals with higher ${}_{PLM}Flow_{Peak}$ response (H-PLM). Individuals in the L-PLM group were classified as the lowest 8 subjects based on ${}_{PLM}Flow_{Peak}$, and the H-PLM were classified as the highest 8. As illustrated in Figure 4A, the H-PLM group exhibited greater ${}_{KE}Flow$ than the L-PLM group, with values at 20 W, 30 W and WR_{max} significantly differing. These findings indicate that endothelial function is not only related to peak flow, but also to flow at submaximal work rates.

Mass Specific

At this point it is important to note that the H-PLM and L-PLM groups pictured in Figure 4A differed in more than just endothelial function, with quadriceps muscle mass and sex being among the more important differences. Thus, a second comparison was made by creating two groups comprised of individuals who exhibited high or low ${}_{PLM}\text{Flow}_{\text{Peak}}$ per kg of quadriceps mass. Prior to quadriceps mass normalization (Figure 4A), sex distribution was 3 males and 5 females in the L-PLM group, and 8 males in the H-PLM group. Once quadriceps mass was normalized, sex distribution was 5 males and 3 females in the L-PLM group, and 6 males and 2 females in the H-PLM group. This helped further isolate endothelial-specific function independent of other influences. As illustrated in Figure 4B, there remained a significant effect of endothelial function between groups, with ${}_{KE}\text{Flow}$ significantly differing at 30 W and peak flow.

At first glance, the relationship between vascular endothelial function, assessed by PLM, and peak exercise blood flow may seem to disagree with previous studies showing no effect of NO synthase inhibition of ${}_{KE}\text{Flow}$. It is important to recognize that differences in exercise intensity separate the current study and several animal-based studies, which implicate a role of NO in exercise blood flow (18,50), with most human NO synthase inhibition studies (8,15). Human studies suggesting that NO-dependent dilation was not essential during exercise were completed at low intensities (20–30 W) (8,15). In contrast, the animal studies that indicated NO inhibition did decrease blood flow were completed at a maximal effort (17,18). Later human studies, utilizing a different mode of exercise (handgrip), found that NO synthase inhibition reduced blood flow by ~20% at high intensities, while having little effect at low intensities (51–53). The present study agrees with the importance of exercise intensity. Results found no relationship between endothelial function and blood flow at low intensities (10–20 W) but found a significant correlation at high intensities (30+ W). Another reason for differing outcomes between studies may be due to limitations in the amount of drugs (i.e., the NO inhibitor N_G-mono-methyl-L-arginine (L-NMMA)) that can safely be administered to humans. A dose within a safe and acceptable range for humans may not be potent enough to elicit a decrease in blood flow, as seen in animals with much greater doses (15,17).

The importance of NO-dependent vasodilation is further supported in studies showing that NO supplementation (via nitrate and nitrite) significantly improved exercise blood flow (54–56). This evidence indicates that vascular endothelial function (NO-dependent dilation) is indeed heavily involved in exercise blood flow. The present study emphasizes the relationship between vascular endothelial function and peak exercise blood flow, suggesting that peak flow may indeed be influenced by NO-dependent dilation, as PLM and ROV assessments of vascular endothelial function are NO-dependent (24,37).

Experimental Considerations

There are some experimental considerations of note in the current study. First, though PLM and ROV are primarily considered assessments of quadriceps muscle blood flow, it is acknowledged that there is some contribution from other muscles in the leg. Second, there may be variation in the ROV single-leg contraction between subjects such that the speed, force of contraction, and anticipation of the kick may all influence quadriceps blood flow measurements. To minimize this effect, subjects were given time to practice the ROV procedure, one kick at a rate of about 90°/s (one extension over the course of 1 s). In the assessment of ${}_{KE}\text{Flow}_{\text{Peak}}$ it was assumed that only knee extensor muscles were used. However, other muscle groups such as core stabilizers or knee flexor muscles may have slightly assisted the subject leading to greater

${}_{KE}Flow_{Peak}$. Nevertheless, familiarization with the exercise helped to minimize this possibility. Vascular density may contribute to the observed relationships between the assessments of endothelial function and flow during knee extension. However, direct assessment of this parameter was outside the scope of this study. Future studies directly assessing the relationship between vascular density, PLM, ROV and ${}_{KE}Flow$ are warranted.

Conclusion

In conclusion, the present study demonstrated that there is a positive, linear correlation between vascular endothelial function and peak exercise leg blood flow. Subjects that had greater endothelial function were able to achieve greater flow during the single leg knee extension exercise. As there are significant differences in leg blood flow at varying work rates between those of high endothelial function and low endothelial function, it appears that the function of the endothelium may have an important role in explaining typical variability in submaximal and peak blood flow observed during exercise (2,3).

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Table 1: Subject Characteristics

Male/Female	12/7
Age (yr)	23 ± 0.57
Height (cm)	172.85 ± 2.04
Weight (kg)	72.8 ± 3.12
BMI (kg·m^{-2})	24.15 ± 0.62
Knee Extension MVC (kg)	66.9 ± 3.66
KE$_{\text{max}}$ (W)	52.6 ± 4.08
Thigh Mass (kg)	5.08 ± 0.38
Quad Mass (kg)	2.11 ± 0.48
Resting Blood Flow (ml·min^{-1})	380.1 ± 27.51
$_{\text{KE}}$Flow$_{\text{Peak}}$ (ml·min^{-1})	4310.47 ± 217.88
$_{\text{PLM}}$Flow$_{\text{Peak}}$ (ml·min^{-1})	1434.6 ± 102.23
$_{\text{ROV}}$Flow$_{\text{Peak}}$ (ml·min^{-1})	1951.45 ± 132.1
FMD (% dilation)	6.15 ± 0.64

Values are ± SE

Table 2: Correlation Between Blood Flow and Vascular Conductance During Knee Extension Exercise and Vascular Endothelial Function

	${}_{\text{KE}}\text{Flow}_{\text{Peak}}$	${}_{\text{KE}}\text{Conductance}_{\text{Peak}}$	${}_{\text{KE}}\text{Flow}_{\text{30 Watts}}$	${}_{\text{KE}}\text{Conductance}_{\text{30 Watts}}$
${}_{\text{KE}}\text{Power}_{\text{Peak}}$ (W)	r = 0.72, *P* = 0.001	r = 0.26, *P* = 0.30	r = 0.46, *P* = 0.05	r = 0.63, *P* = 0.006
Quadriceps Mass (kg)	r = 0.64, *P* = 0.001	r = 0.51, *P* = 0.03	r = 0.46, *P* = 0.05	r = 0.56, *P* = 0.01
BMI ($\text{kg}\cdot\text{m}^{-2}$)	r = 0.72, *P* = 0.001	r = 0.28, *P* = 0.27	r = 0.49, *P* = 0.03	r = 0.64, *P* = 0.006
Gender (F = 1, M = 2)	r = 0.77, *P* = 0.001	r = 0.46, *P* = 0.06	r = 0.56, *P* = 0.01	r = 0.65, *P* = 0.004
${}_{\text{PLM}}\text{Flow}_{\text{Peak}}$ ($\text{ml}\cdot\text{min}^{-1}$)	r = 0.77, *P* = 0.001	r = 0.57, *P* = 0.02	r = 0.47, *P* = 0.04	r = 0.52, *P* = 0.03
${}_{\text{PLM}}\text{Flow}_{\text{AUC}}$ (ml)	r = 0.72, P = 0.001	r = 0.41, P = 0.10	r = 0.40, *P* = 0.09	r = 0.29, *P* = 0.25
${}_{\text{PLM}}\text{Conductance}_{\text{Peak}}$ ($\text{ml}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}$)	r = 0.71, P = 0.002	r = 0.51, P = 0.04	r = 0.36, *P* = 0.16	r = 0.42, *P* = 0.10
${}_{\text{PLM}}\text{Conductance}_{\text{AUC}}$ ($\text{ml}\cdot\text{mmHg}^{-1}$)	r = 0.54, P = 0.03	r = 0.25, P = 0.36	r = 0.10, *P* = 0.72	r = 0.09, *P* = 0.77
${}_{\text{ROV}}\text{Flow}_{\text{Peak}}$ ($\text{ml}\cdot\text{min}^{-1}$)	r = 0.69, *P* = 0.001	r = 0.60, *P* = 0.01	r = 0.67, *P* = 0.03	r = 0.64, *P* = 0.005
${}_{\text{ROV}}\text{Flow}_{\text{AUC}}$ (ml)	r = 0.16, *P* = 0.55	r = 0.31, *P* = 0.22	r = 0.24, *P* = 0.35	r = 0.03, *P* = 0.99
${}_{\text{ROV}}\text{Conductance}_{\text{Peak}}$ ($\text{ml}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}$)	r = 0.13, *P* = 0.62	r = −0.15, *P* = 0.55	r = 0.21, *P* = 0.40	r = 0.15, *P* = 0.57
${}_{\text{ROV}}\text{Conductance}_{\text{AUC}}$ ($\text{ml}\cdot\text{mmHg}^{-1}$)	r = 0.21, *P* = 0.40	r = 0.19, *P* = 0.46	r = 0.25, *P* = 0.31	r = 0.11, *P* = 0.69
FMD (% dilation)	r = −0.02, *P* = 0.86	r = −0.08, *P* = 0.74	r = −0.12, *P* = 0.62	r = −0.42, *P* = 0.09
${}_{\text{FMD}}\text{Flow}_{\text{Peak}}$ ($\text{ml}\cdot\text{min}^{-1}$)	r = 0.55, *P* = 0.01	r = 0.48, *P* = 0.04	r = 0.55, *P* = 0.01	r = 0.50, *P* = 0.04

${}_{\text{KE}}\text{Flow}_{\text{Peak}}$, peak blood flow during knee extension exercise;
${}_{\text{KE}}\text{Flow}_{30}$, blood flow during knee extension exercise at 30 W;
${}_{\text{KE}}\text{Conductance}_{\text{Peak}}$, peak vascular conductance during knee extension exercise;
${}_{\text{KE}}\text{Conductance}_{30}$, vascular conductance during knee extension at 30 W;
${}_{\text{KE}}\text{Power}_{\text{Peak}}$, knee extension maximum watts;
BMI, Body mass index;
${}_{\text{PLM}}\text{Flow}_{\text{Peak}}$, peak blood flow during passive leg movement;
${}_{\text{PLM}}\text{Flow}_{\text{AUC}}$, area under the curve for blood flow during passive leg movement;
${}_{\text{PLM}}\text{Conductance}_{\text{Peak}}$, peak vascular conductance during passive leg movement;
${}_{\text{PLM}}\text{Conductance}_{\text{AUC}}$, area under the curve for vascular conductance during passive leg movement;
${}_{\text{ROV}}\text{Flow}_{\text{Peak}}$, peak blood flow during rapid onset vasodilation;
${}_{\text{ROV}}\text{Flow}_{\text{AUC}}$, area under the curve for blood flow during rapid onset vasodilation;
${}_{\text{ROV}}\text{Conductance}_{\text{Peak}}$, peak vascular conductance during rapid onset vasodilation;
${}_{\text{ROV}}\text{Conductance}_{\text{AUC}}$, area under the curve for vascular conductance during rapid onset vasodilation;
FMD, flow-mediated dilation as a % change;
${}_{\text{FMD}}\text{Flow}_{\text{Peak}}$, peak blood flow during flow-mediated dilation.

Table 3: Correlation Between Blood Flow During Knee Extension Exercise and Vascular Endothelial Function Normalizing for Quadriceps Mass

	${}_{\mathrm{KE}}\mathrm{Flow}_{\mathrm{Peak}}$	${}_{\mathrm{KE}}\mathrm{Flow}_{30\ \mathrm{Watts}}$
${}_{\mathrm{KE}}\mathrm{Power}_{\mathrm{Peak}}$ (W)	r = –0.25, *P* = 0.29	r = –0.33, *P* = 0.17
BMI (kg·m^{-2})	r = 0.10, *P* = 0.69	r = –0.30, *P* = 0.21
Gender (F = 1, M = 2)	r = 0.13, *P* = 0.59	r = –0.27, *P* = 0.26
${}_{\mathrm{PLM}}\mathrm{Flow}_{\mathrm{Peak}}$ (ml·min^{-1}·kg^{-1})	r = 0.61, *P* = 0.001	r = 0.32, *P* = 0.18
${}_{\mathrm{PLM}}\mathrm{Flow}_{\mathrm{AUC}}$ (ml·kg^{-1})	r = 0.54, *P* = 0.03	r = 0.18, *P* = 0.46
${}_{\mathrm{ROV}}\mathrm{Flow}_{\mathrm{Peak}}$ (ml·min^{-1}·kg^{-1})	r = 0.51, *P* = 0.05	r = 0.38, *P* = 0.11
${}_{\mathrm{ROV}}\mathrm{Flow}_{\mathrm{AUC}}$ (ml·kg^{-1})	r = 0.41, *P* = 0.08	r = 0.42, *P* = 0.08
FMD (% dilation·kg^{-1})	r = 0.45, *P* = 0.05	r = 0.58, *P* = 0.01
${}_{\mathrm{FMD}}\mathrm{Flow}_{\mathrm{Peak}}$ (ml·min^{-1}·kg^{-1})	r = 0.24, *P* = 0.33	r = 0.42, *P* = 0.07

Normalizing for quadriceps mass by dividing by mass in kg.
${}_{\mathrm{KE}}\mathrm{Flow}_{\mathrm{Peak}}$, peak blood flow during knee extension exercise;
${}_{\mathrm{KE}}\mathrm{Flow}_{30}$, blood flow during knee extension exercise at 30 W;
${}_{\mathrm{KE}}\mathrm{Power}_{\mathrm{Peak}}$, knee extension maximum watts;
BMI, Body mass index;
${}_{\mathrm{PLM}}\mathrm{Flow}_{\mathrm{Peak}}$, peak blood flow during passive leg movement;
${}_{\mathrm{PLM}}\mathrm{Flow}_{\mathrm{AUC}}$, area under the curve for blood flow during passive leg movement;
${}_{\mathrm{ROV}}\mathrm{Flow}_{\mathrm{Peak}}$, peak blood flow during rapid onset vasodilation;
${}_{\mathrm{ROV}}\mathrm{Flow}_{\mathrm{AUC}}$, area under the curve for blood flow during rapid onset vasodilation;
FMD, flow-mediated dilation as a % change;
${}_{\mathrm{FMD}}\mathrm{Flow}_{\mathrm{Peak}}$, peak blood flow during flow-mediated dilation.

Table 4: Partial Correlation Between Knee Extension Peak Exercise Blood Flow and Various Indices of Endothelial Function when Controlling for A) Quadriceps Mass and B) Power Output During Knee Extension

A	$_{KE}Flow_{Peak}$
$_{PLM}Flow_{Peak}$ (ml·min^{-1}·kg^{-1})	r = 0.64, P = 0.004
$_{PLM}Flow_{AUC}$ (ml·kg^{-1})	r = 0.62, P = 0.007
$_{ROV}Flow_{Peak}$ (ml·min^{-1}·kg^{-1})	r = 0.57, P = 0.01
$_{ROV}Flow_{AUC}$ (ml·kg^{-1})	r = 0.37, P = 0.13
FMD (% dilation·kg^{-1})	r = 0.38, P = 0.12
$_{FMD}Flow_{Peak}$ (ml·min^{-1}·kg^{-1})	r = 0.29, P = 0.25

B	$_{KE}Flow_{Peak}$
$_{PLM}Flow_{Peak}$ (ml·min^{-1}·W^{-1})	r = 0.70, P = 0.001
$_{PLM}Flow_{AUC}$ (ml·W^{-1})	r = 0.63, P = 0.005
$_{ROV}Flow_{Peak}$ (ml·min^{-1}·W^{-1})	r = 0.61, P = 0.005
$_{ROV}Flow_{AUC}$ (ml·W^{-1})	r = 0.25, P = 0.32
FMD (% dilation·W^{-1})	r = 0.10, P = 0.70
$_{FMD}Flow_{Peak}$ (ml·min^{-1}·W^{-1})	r = 0.36, P = 0.14

(**A**) Controlling for quadriceps mass and (**B**) controlling for power output by dividing by watts.
$_{KE}Flow_{Peak}$, peak blood flow during knee extension exercise;
$_{PLM}Flow_{Peak}$, peak blood flow during passive leg movement;
$_{PLM}Flow_{AUC}$, area under the curve for blood flow during passive leg movement;
$_{ROV}Flow_{Peak}$, peak blood flow during rapid onset vasodilation;
$_{ROV}Flow_{AUC}$, area under the curve for blood flow during rapid onset vasodilation;
FMD, flow-mediated dilation as a % change;
$_{FMD}Flow_{Peak}$, peak blood flow during flow-mediated dilation.

Table 5: Femoral Artery Blood Flow During Knee Extension in Individuals of Low and High Peak PLM-Induced Hyperemia in (A) Absolute Values and (B) Normalizing for Quadriceps Mass

A	Low PLM Responders	High PLM Responders
Gender (M/F)	3/5	8/0*
BMI (kg·m^{-2})	22.8 ± 0.7	25.8 ± 0.7*
Quadriceps Mass (kg)	1.9 ± 0.1	2.5 ± 0.2*
${}_{KE}\text{Power}_{Peak}$ (W)	46.3 ± 5.6	65.0 ± 5.0*
${}_{PLM}\text{Flow}_{Peak}$ (ml·min^{-1})	1031 ± 45	1859 ± 107*
${}_{PLM}\text{Flow}_{AUC}$ (ml)	248 ± 50	715 ± 67*
${}_{ROV}\text{Flow}_{Peak}$ (ml·min^{-1})	1798 ± 150	2528 ± 209*
${}_{ROV}\text{Flow}_{AUC}$ (ml)	380 ± 36	377 ± 45
FMD (% dilation)	7.1 ± 0.9	5.1 ± 1.0
FMDFlowPeak (ml·min^{-1})	1618 ± 83	1913 ± 231

B	Low PLM Responders	High PLM Responders
Gender (M/F)	5/3	6/2
BMI (kg·m^{-2})	24.2 ± 0.9	24.6 ± 1.4
Quadriceps Mass (kg)	2.3 ± 0.2	2.0 ± 0.1
${}_{KE}\text{Power}_{Peak}$ (W·kg^{-1})	25.5 ± 2.1	26.2 ± 2.8
${}_{PLM}\text{Flow}_{Peak}$ (ml·min^{-1}·kg^{-1})	521 ± 29	835 ± 34*
${}_{PLM}\text{Flow}_{AUC}$ (ml·kg^{-1})	167 ± 24	290 ± 18*
${}_{ROV}\text{Flow}_{Peak}$ (ml·min^{-1}·kg^{-1})	880 ± 72	930 ± 77
${}_{ROV}\text{Flow}_{AUC}$ (ml·kg^{-1})	167 ± 18	152 ± 22
FMD (% dilation·kg^{-1})	2.2 ± 0.8	3.3 ± 0.3
${}_{FMD}\text{Flow}_{Peak}$ (ml·min^{-1}·kg^{-1})	797 ± 74	819 ± 54

Low/high PLM responders are 8 subjects with lowest/highest peak blood flow during passive leg movement.
BMI, Body mass index;
${}_{KE}\text{Power}_{Peak}$, knee extension maximum watts;
${}_{PLM}\text{Flow}_{Peak}$, peak blood flow during passive leg movement;
${}_{PLM}\text{Flow}_{AUC}$, area under the curve for blood flow during passive leg movement;
${}_{ROV}\text{Flow}_{Peak}$, peak blood flow during rapid onset vasodilation;
${}_{ROV}\text{Flow}_{AUC}$, area under the curve for blood flow during rapid onset vasodilation;
FMD, flow-mediated dilation as a % change;
${}_{FMD}\text{Flow}_{Peak}$, peak blood flow during flow-mediated dilation.
*$P < 0.05$ vs Low PLM Responders.

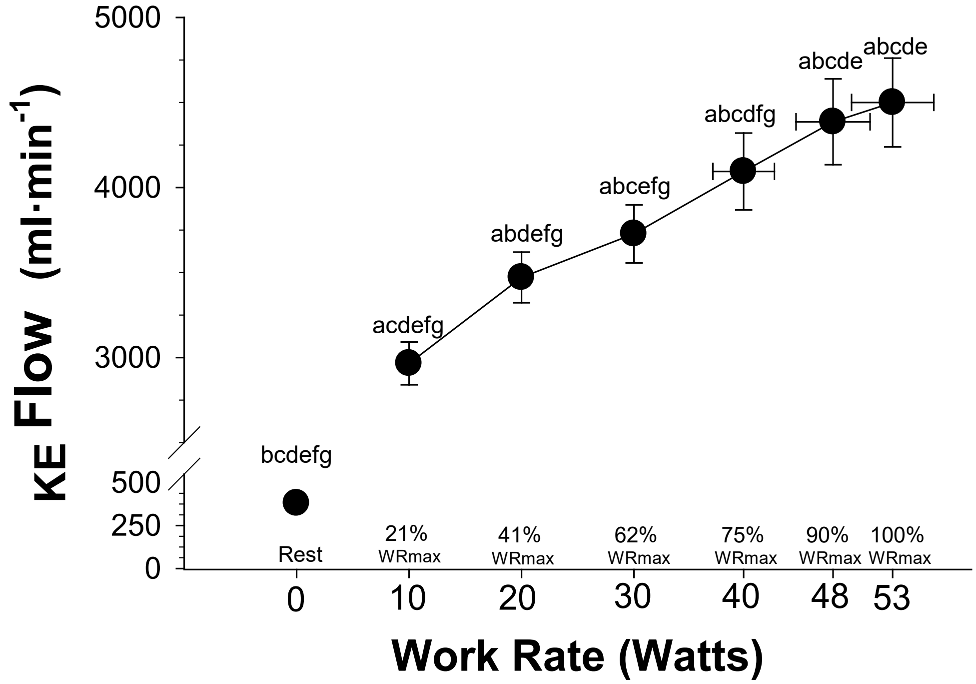

Figure 1: Average femoral artery blood flow during knee extension exercise ($_{KE}$Flow) conducted at 10, 20, 30 W, and 75%, 90%, 100% WR_{max}.

a: significantly different from resting
b: significantly different from 10 W
c: significantly different from 20 W
d: significantly different from 30 W
e: significantly different from 75% WR_{max}
f: significantly different from 90% WR_{max}
g: significantly different from 100% WR_{max}

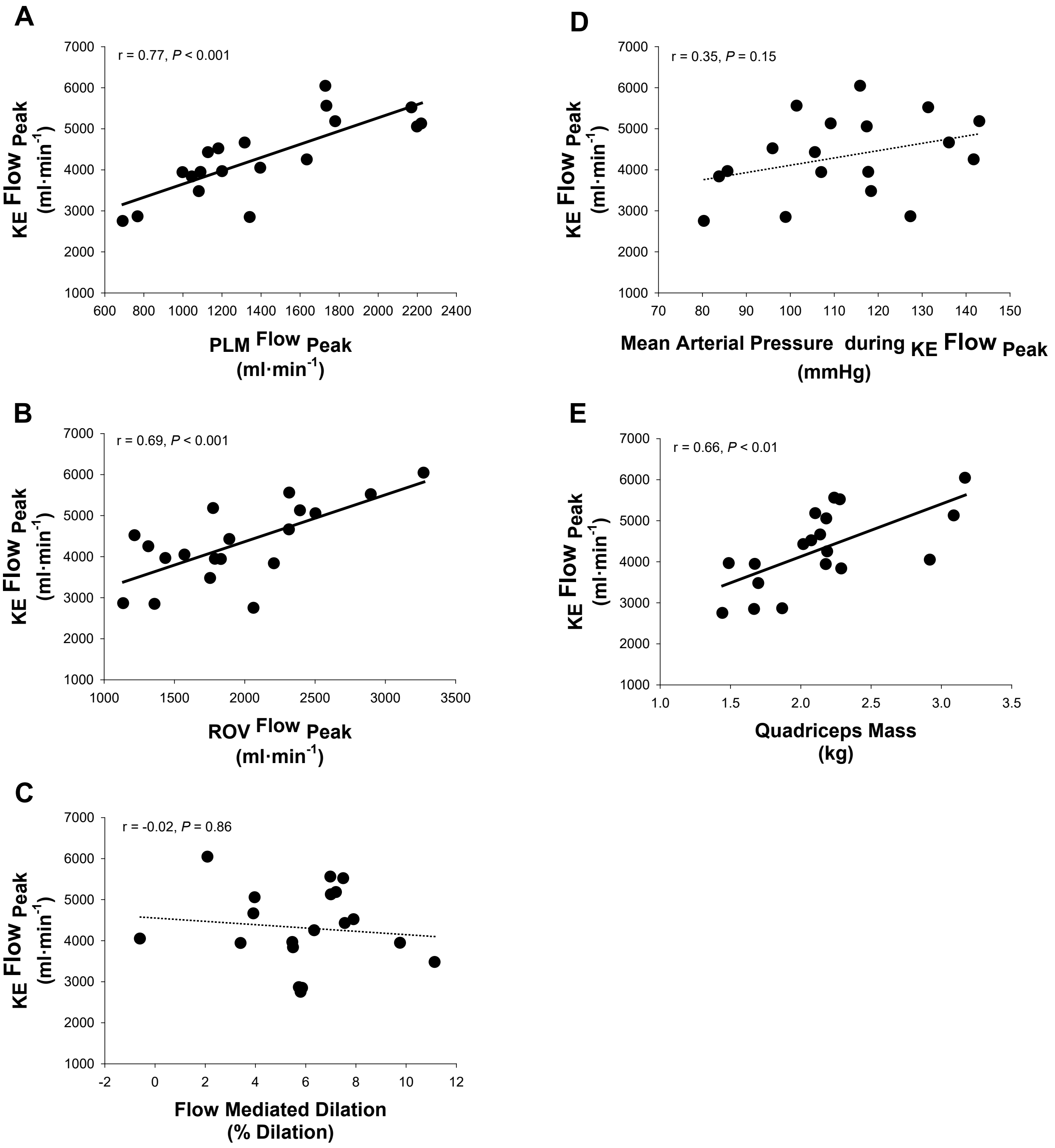

Figure 2: The relationship between different indices of endothelial function and peak blood flow during knee extension exercise ($_{\mathrm{KE}}$Flow$_{\mathrm{Peak}}$).

A Peak blood flow during passive leg movement ($_{\mathrm{PLM}}$Flow$_{\mathrm{Peak}}$) and $_{\mathrm{KE}}$Flow$_{\mathrm{Peak}}$.

B Peak blood flow during rapid onset vasodilation ($_{\mathrm{ROV}}$Flow$_{\mathrm{Peak}}$) and $_{\mathrm{KE}}$Flow$_{\mathrm{Peak}}$.

C Percent dilation during flow-mediated dilation and $_{\mathrm{KE}}$Flow$_{\mathrm{Peak}}$.

D Mean arterial pressure during knee extension exercise and $_{\mathrm{KE}}$Flow$_{\mathrm{Peak}}$.

E Quadriceps mass and $_{\mathrm{KE}}$Flow$_{\mathrm{Peak}}$. Note that bold lines represent a significant correlation.

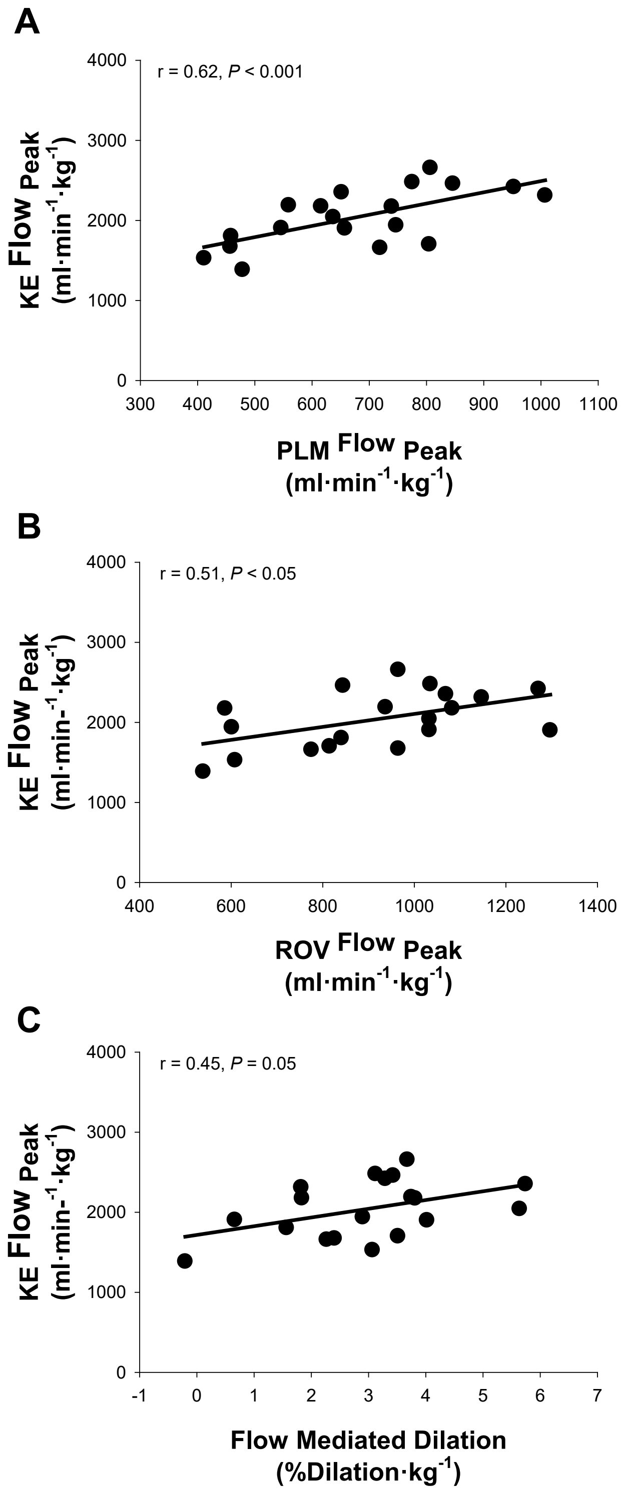

Figure 3: The relationship between different indices of endothelial function and peak blood flow during knee extension exercise (KEFlow$_{Peak}$) when normalizing for quadriceps mass.

A Peak blood flow during passive leg movement (PLMFlow$_{Peak}$) and KEFlow$_{Peak}$.

B Peak blood flow during rapid onset vasodilation (ROVFlow$_{Peak}$) and KEFlow$_{Peak}$.

C Percent dilation during flow-mediated dilation and KEFlow$_{Peak}$.

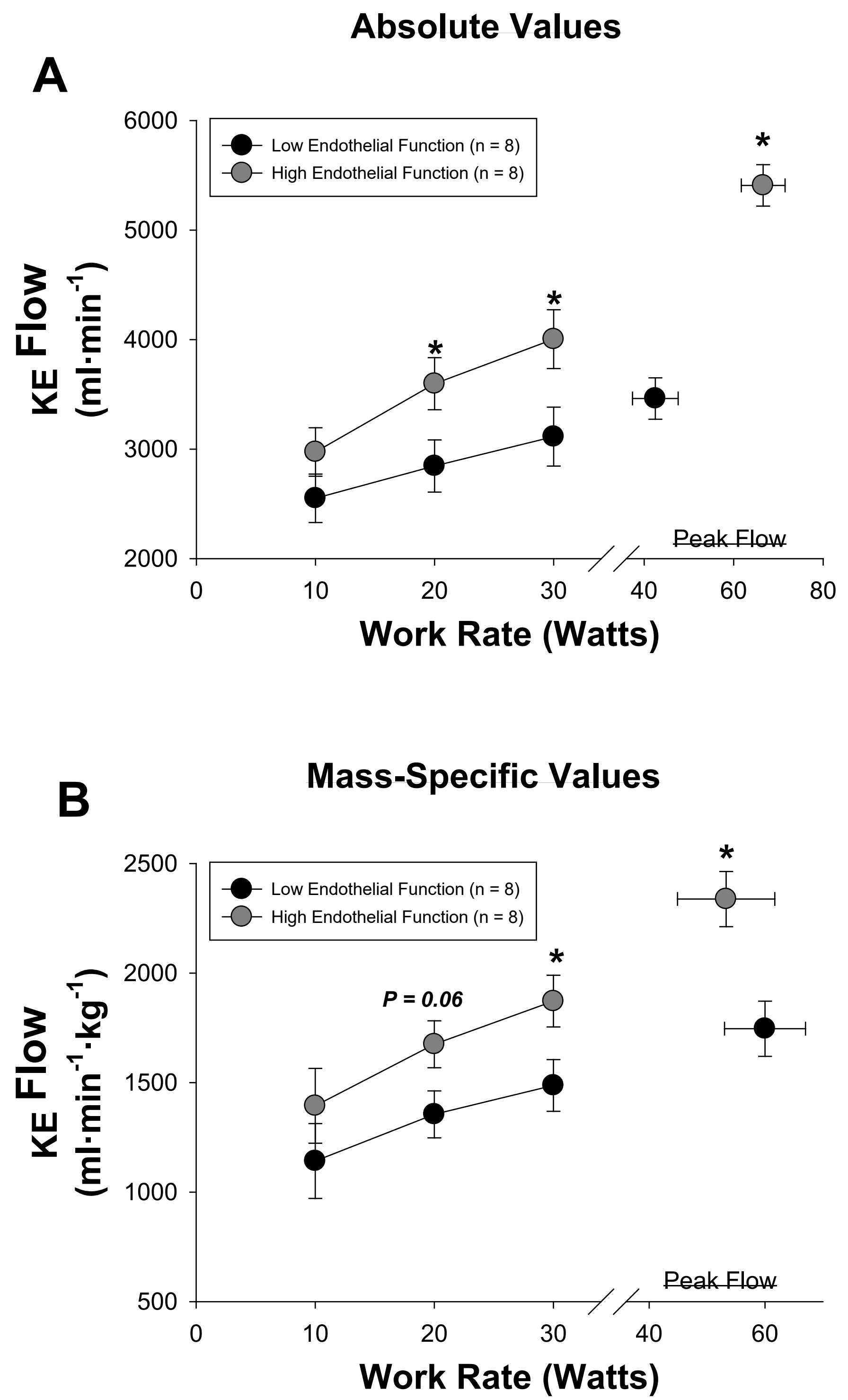

Figure 4: Femoral artery blood flow during knee extension exercise in individuals who had low and high peak PLM-induced hyperemia.

A Absolute blood flow values

B Normalizing for quadriceps mass. Peak flow was the highest blood flow (ml·min^{-1}) achieved at highest watts per subject (horizontal error bars)

*$P < 0.05$ vs Low PLM Responders