Human muscle length-dependent changes in blood flow

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McDaniel J, Ives SJ, Richardson RS. Human muscle length-dependent changes in blood flow. J Appl Physiol 112: 560–565, 2012. First published December 1, 2011; doi:10.1152/japplphysiol.01223.2011.—Although a multitude of factors that influence skeletal muscle blood flow have been extensively investigated, the influence of muscle length on limb blood flow has received little attention. Thus the purpose of this investigation was to determine if cyclic changes in muscle length influence resting blood flow. Nine healthy men (28 ± 4 yr of age) underwent a passive knee extension protocol during which the subjects’ knee joint was passively extended and flexed through 100–180° knee joint angle at a rate of 1 cycle per 30 s. Femoral blood flow, cardiac output (CO), heart rate (HR), stroke volume (SV), and mean arterial pressure (MAP) were continuously recorded during the entire protocol. These measurements revealed that slow passive changes in knee joint angle did not have a significant influence on HR, SV, MAP, or CO; however, net femoral blood flow demonstrated a curvilinear increase with knee joint angle ($r^2 = 0.98$) such that blood flow increased by ~90% (125 ml/min) across the 80° range of motion. This net change in blood flow was due to a constant antegrade blood flow across knee joint angle and negative relationship between retrograde blood flow and knee joint angle ($r^2 = 0.98$). Thus, despite the absence of central hemodynamic changes and local metabolic factors, blood flow to the leg was altered by changes in muscle length. Therefore, when designing research protocols, researchers need to be cognizant of the fact that joint angle, and ultimately muscle length, influence limb blood flow.

Skeletal muscle capillaries are well secured to surrounding tissue, thus muscle lengthening and shortening results in stretching and compressing the vasculature, which alters resistance and subsequently alters blood flow through this network. Poole et al. (29) reported that capillary tortuosity decreased with increases in sarcomere length up to 2.6 μm, at which point further increases in sarcomere length reduced mean capillary diameter. As a result of these changes in capillary geometry, blood flow decreased by 40%. Thus it is likely that as the muscle is stretched the initial decrease in tortuosity increases vascular conductance, while further stretching of the muscle and narrowing of the vessels results in decreased vascular conductance. There is also evidence that sympathetic nerve activity and vasomotor tone are influenced by muscle length such that increasing muscle length results in increased sympathetic tone (35). Additionally, rapid changes in muscle length can increase mechanoreceptor afferent feedback to the cardiovascular control center, increasing heart rate and ultimately cardiac output (14, 22, 23, 33, 34). Thus, although changes in muscle length may alter blood flow through multiple mechanisms, using very slow passive muscle movement, below that which would stimulate the mechanoreceptors, may allow an examination of the influence of changes in muscle length on blood flow in humans without the increase in cardiac output that is observed at faster rates of movement.

Thus, using a reductionist approach, the purpose of this investigation was to determine if slow changes in muscle length in humans would 1) elicit a cardiovascular response and 2) influence peripheral skeletal muscle blood flow. Specifically, we aimed to determine if femoral blood flow is affected by slow passive extension and flexion of the knee joint. In regards to this model we presume that changes in the length of the knee extensors would predominate over the competing effects of the simultaneous and opposite changes in knee flexors owing to the 2.5-fold greater mass of the knee extensors relative to the flexors (3, 5) and the greater length changes the extensors undergo during knee extension/flexion compared with the flexors (13). Thus we hypothesized that during this slow passive movement femoral blood flow will increase as knee joint angle increases, a surrogate for muscle length, despite an unchanged cardiac output.

METHODS

Subjects and General Procedures

Nine healthy men (28 ± 4 yr, 178 ± 8 cm, 87 ± 21 kg) participated in the current study. The protocol was approved by the Institutional Review Boards of the University of Utah and the Salt Lake City Veterans Affairs Medical Center, and written informed consent was obtained from all subjects prior to their inclusion in the study. All

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studies were performed in a thermoneutral environment (22°C). Subjects refrained from exercise for 24 h before data collection.

Passive Knee Extension and Flexion

Prior to the start of data collection, subjects lay supine for 20 min. During this period subjects were equipped with ECG electrodes and a finometer finger pressure cuff. In addition, the subjects were outfitted with a knee brace equipped with a position sensor that supplied real-time knee angle feedback that ensured a consistent range of motion during slow passive leg movement and served as a surrogate measure of thigh muscle length changes. One minute before the start of each slow passive exercise protocol, a pneumatic cuff, placed distal to the knee on the passively moved leg, was inflated to 250 mmHg, eliminating blood flow to the lower leg. The cuff, which remained inflated throughout the entire protocol, eliminated fluctuations in blood flow to the lower leg as a consequence of partial occlusion of the popliteal artery during knee flexion as well as movement-related changes in gravitational and centrifugal forces. The protocol itself consisted of five cycles of slow passive knee extension/flexion (30 s per flexion/extension cycle) through the range of motion defined by 100 and 180° knee joint angle (full knee extension = 180°). Prior to the start and throughout the protocol subjects were encouraged to remain passive and resist any urge to assist with leg movement. In the rare instance that a subject assisted with or resisted the movement, the protocol was terminated and repeated after 10 min of recovery.

Measurements

Femoral blood flow. Measurements of femoral arterial blood velocity and vessel diameter were taken in the passively moved leg distal to the inguinal ligament and proximal to the bifurcation of the superficial and deep femoral artery with a Logic 7 ultrasound system (General Electric Medical Systems, Milwaukee, WI). The Logic 7 was equipped with a linear array transducer operating at an imaging frequency of 14 MHz. Vessel diameter was determined at a perpendicular angle along the central axis of the scanned area. Blood velocity was obtained using the same transducers with a Doppler frequency of 5 MHz. All blood velocity measurements were obtained with the probe appropriately positioned to maintain an insonation angle of 60° or less. The sample volume was maximized according to vessel size and was centered within the vessel based on real-time ultrasound visualization. Arterial diameter was measured and angle corrected, and intensity weighted mean velocity (Vmean) values were then calculated using commercially available software (Logic 7). By using arterial diameter and Vmean, blood flow in the femoral artery was calculated as: blood flow = Vmean(r(vessel diameter/2)^2) × 60, where blood flow is in milliliters per minute.

Central variables. HR, SV, CO, and MAP were determined with a Finometer (Finapres Medical Systems BV, Amsterdam, the Netherlands). SV was calculated using the Modelflow method (6, 11, 32), which includes age, sex, height, and weight in its algorithm (Beatscope version 1.1; Finapres Medical Systems BV). CO was then calculated as the product of HR and SV. Vascular conductance within the passively moved leg was calculated as leg blood flow/MAP (ml·min^{-1}·mmHg^{-1}).

Knee joint angle. During each protocol, knee joint angle of the passive leg was continuously recorded using a Vishay Spectrol 360 degree Smart Position Sensor (Vashay Intertechnology, Malvern, PA) mounted on a BREG X2K knee brace (BREG, Vista, CA) worn by the subjects.

Data acquisition. Throughout each protocol, signals reflecting HR, SV, CO, MAP, and knee joint angle underwent analog to digital conversion and were simultaneously acquired (200 Hz) using commercially available AcqKnowledge data-acquisition software (Biopac Systems, Goleta, CA). In addition, the antegrade and retrograde audio signals from the Doppler ultrasound system were acquired (10,000 Hz) to serve as a qualitative indicator of blood velocity changes and to ensure accurate temporal alignment of blood velocity measurements obtained from the Doppler system and other variables collected (i.e., HR, SV, CO, MAP, as well as the knee joint angle).

Data Analysis

All central variables (HR, SV, CO, MAP) and femoral blood flow were recorded on a beat by beat basis. These variables were initially partitioned into three ranges of knee joint angle (lower 15%, middle 15%, and upper 15% range). Repeated-measures ANOVA was performed on HR, SV, CO, MAP, net blood flow, antegrade blood flow, retrograde blood flow, and conductance across these three ranges of joint angle. Post hoc comparisons (with a Bonferroni adjustment) were used to determine where differences existed if the overall ANOVA was significant. Regression analyses were performed on a group and individual basis for net blood flow, antegrade blood flow, retrograde blood flow, as well as the change in blood flow relative to blood flow at 100° that was averaged into 5° increments across all five passive knee extension/flexion cycles. Initially the regression analysis was run on the average data for all subjects. If this analysis yielded a significant linear relationship, then a linear analysis was also applied to all the individual data sets for that variable; however, if this analysis revealed a quadratic relationship, as indicated by a statistically significant squared term, then a quadratic analysis was also applied to the individual data sets for that variable. The alpha level for all comparisons was 0.05, and all data are presented as mean ± SD.

RESULTS

Central Variables

Change in knee joint angle during slow cyclic knee extension and flexion did not have a significant effect on HR, SV, or CO (Table 1). MAP, although not statistically significant, tended to be greater as knee angle decreased (P = 0.06; Table 1).

Blood Flow and Vascular Conductance During Slow Passive Movement

Relative femoral blood flow and knee joint angle during slow knee flexion/extension from one representative subject is presented in Fig. 1A. Relative to blood flow at the lower range of knee joint angle, blood flow increased by ~32% and ~51% at the middle and upper range of knee joint angles, respectively (Fig. 2). Retrograde blood flow decreased across all three ranges of knee joint angle (Fig. 2). Specifically, retrograde blood flow decreased by 20% when the knee was passively extended from the lower to the middle range of knee joint angle and by an additional 15% as it continued through to the upper range of knee joint angle. Unlike net and retrograde blood flow, there was no difference in antegrade blood flow across the

| Table 1. HR, SV, MAP, and CO across three ranges of knee joint angle |
|---------------|---------------|---------------|
|                | Lower 15%    | Middle 15%   | Upper 15%    |
| HR, beats/min  | 58 ± 7       | 59 ± 8       | 59 ± 7       |
| SV, ml         | 111 ± 20     | 111 ± 21     | 111 ± 21     |
| MAP, mmHg      | 88 ± 9       | 87 ± 9       | 86 ± 8       |
| CO, l/min      | 6.5 ± 1.7    | 6.5 ± 1.7    | 6.6 ± 1.7    |
| Conductance, ml·min^{-1}·mmHg^{-1} | 2.4 ± 0.8±   | 3.2 ± 1.1±   | 3.6 ± 1.1±   |

Values are means ± SD. HR, heart rate; SV, stroke volume; MAP, mean arterial pressure; CO, cardiac output. *Significant difference from the lower 15% range of knee joint. †Significant difference from the middle 15% range of knee joint.
Regression analysis revealed that there was a quadratic relationship between net femoral blood flow and knee joint angle: femoral blood flow \(= -234 + 6 (k) - 0.0144(k)^2; \quad r^2 = 0.98 \) where \(k\) = knee joint angle (Fig. 3A). This equation reveals an increase of 125.4 ml/min across the entire range of motion used in this investigation (from 100 to 180°) or an average of 1.6 ml/min/°. The average \(r^2\) for all nine individual regression equations equaled 0.83 ± 0.13 (range: 0.61 to 0.98).

Expressing the change in net blood flow at each 5° knee joint angle, relative to blood flow at 100° knee joint angle, also revealed a significant quadratic relationship: relative change in blood flow \(= -342 + 4.55 (k) - 0.012 (k)^2 \quad (r^2 = 0.97; \text{Fig. 1B}).\)

Thus blood flow increased by 90% as the knee was extended from 100 to 180° (note: this difference is larger than that described with the repeated-measures analysis because it focuses on the extreme joint angles, which are not averaged into the three knee joint ranges, thus maximizing the effect size).

Antegrade and retrograde blood flow regression analysis partially agreed with the repeated-measures analysis of the three ranges of knee joint angle (lower, middle, and upper). Specifically, when all individual antegrade data were combined into one regression, a significant positive relationship still existed \(r^2 = 0.62\) and slope \(-0.37\) ml/min/°. However, three ranges of knee joint angle \(P = 0.48\). In addition, conductance increased by \(-30\%\) as the knee was extended from the lower to middle range of knee joint angle and by an additional 16% as it continued through to the upper range of knee joint angle (Table 1).
individual significant positive linear relationships between antegrade blood flow and knee joint angle were only evident in 5 of the 9 subjects with an average $r^2 = 0.54 \pm 0.18$ and average slope = 0.74 ± 0.35 ml/min°. In regards to retrograde blood flow, when all individual data were combined into one regression, a significant quadratic relationship was evident ($r^2 = 0.98$). Individual quadratic regression analyses for retrograde blood flow were more consistent than the antegrade blood flow (average $r^2 = 0.82$ and a range of 0.55 to 0.98; Fig. 3C).

DISCUSSION

Although a multitude of factors that influence skeletal muscle blood flow have been extensively investigated, the influence of muscle length on blood flow in humans has received little attention. Thus in the present study we sought to determine the influence of slow changes in knee joint angle, a surrogate for quadriceps muscle length, on femoral blood flow. The major finding of this study was that, at rest, femoral blood flow increased by ~90% as knee joint angle increased from 100 to 180°. As central parameters including CO, MAP, SV, and HR did not change and the cuff occlusion below the knee eliminated the influence of both gravitational forces and partial popliteal artery occlusion from influencing blood flow, the change in femoral artery blood flow most likely resulted from peripheral factors, including changes in capillary tortuosity and decreased diameter associated with the changes in muscle length. These results are not only important for the understanding of blood flow distribution, but also have important implications for the methodological approach employed by investigators measuring resting blood flow in human limbs.

Blood Flow and Joint Angle

The data acquired during this investigation reveal that femoral artery blood flow is clearly altered by knee joint angle. As illustrated in Fig. 1A, during five cycles of slow passive knee extension/flexion the second-by-second changes in blood flow directly reflect changes in knee joint angle. Two subsequent analyses were performed on these data. Initially both central and peripheral variables collected during the slow passive movement were divided into three ranges: upper, middle, and lower 15% of the knee joint angle. This analytical approach revealed that femoral artery blood flow increased as the knee was extended from the lower to the middle and upper range of knee joint angle (Fig. 2) and that factors underlying this response are peripheral in nature as passive knee extension did not increase HR, SV, or CO (Table 1). Although not statistically significant the tendency for MAP to decrease as the knee joint angle was increased contributed to the parallel increase in vascular conductance (Table 1). Additionally, the decrease in retrograde blood flow at greater knee joint angles can be interpreted to indicate that the microvascular network was capable of accepting more blood as the knee joint was extended (Fig. 2). In contrast, the constancy of the antegrade blood flow, perhaps predominantly determined by the capacitance of the large arteries, was unaffected by joint angle (Fig. 2).

Regression analysis, which includes data throughout the entire range of motion, also lead to the conclusion that femoral artery blood flow at rest was, at least partially, dictated by knee joint angle (Figs. 1B and 3). On the basis of the quadratic relationship between net femoral blood flow and knee joint angle femoral blood flow would be expected to increase by ~125 ml/min (90%) across the 80° range of motion used in the current investigation. The changes in conductance within the vascular network are most likely due to muscle length-dependent changes in capillary tortuosity and vessel diameter. Specifically, Mathieu-Costello (21) determined that muscle sarcomereme length, not fiber type or muscle architecture, determines the degree of vessel tortuosity. At short muscle lengths, the capillaries are highly tortuous and as muscle length increases the vessels become aligned with the longitudinal axis of the muscle fiber and tortuosity decreases. Further lengthening of the muscle results in narrowing of the capillary diameter (24, 28). Although we did not observe a clear local maximum blood flow, as would be expected based on Mathieu-Costello’s data, the documented relationship between knee joint angle and blood flow was indeed curvilinear in nature (Figs. 1 and 3). If, however, the quadriceps were allowed to become shorter than the length at a knee angle of 180°, we would hypothesize that the increase in vessel tortuosity would have a greater influence at such short lengths and ultimately additionally impede blood flow. Although, to some extent, the current data, collected in vivo in humans, support these prior morphometric measurements in rats, further comparison would not be appropriate due to the dissimilarities in species, type of muscle, muscle architecture, and range of motion used in prior studies and the present investigation.

Poole et al. (29) used an in situ technique to determine whether these muscle length-dependent changes in capillary alignment and diameter are associated with changes in red blood cell flow dynamics. They concluded that in rat spinotrapezius muscle blood flow decreased by 40% as the muscle length increased across the largest range of motion. More recently, Mizuno et al. (25) reported a greater pressor response during submaximal isometric knee extension at 90° degrees compared with 130° (25). Although not measured in their investigation, they attributed the greater pressor response to reduced skeletal muscle perfusion at 90°. Mizuno et al. (25) concluded that the decreased perfusion resulted in a reduction in tissue oxygenation and subsequent afferent feedback to the cardiovascular centers. The data from this investigation, albeit in relaxed muscle, support their conclusion as muscle perfusion was drastically reduced with greater flexion of the knee joint. In addition to the muscle length changes in capillary diameter, it seems likely that stretch-induced increases in intramuscular pressure may also impinge on arterioles and venules (4, 16, 30). Thus, as the knee joint flexes from 180 to 90°, the lengthening of the quadriceps muscle group increases vascular resistance and subsequently decreases net femoral blood flow. Experimentally, such muscle length-dependent changes in skeletal muscle blood flow could have a significant impact on the dispersion of blood flow due, for example, to the infusion of a vasoactive substance or the magnitude of increase in blood flow if pre- and postassessments are not made at the same joint angle.
Perivascular Sympathetic Nerve Stimulation and Muscle Length

To elucidate additional mechanisms that may be responsible for the change in blood flow with muscle length, Welsh and Segal (35) investigated whether sympathetic nerve activity coordinates muscle length-dependent changes in hamster retractor muscle blood flow. Similarly to the previously mentioned investigations, their data also revealed that passive lengthening of the muscle appeared to result in actual vasocostriction and reduction in blood flow by 50–60%. The infusion of nitroprusside attenuated the vasoconstriction induced by muscle lengthening, suggesting that factors other than muscular pressure and vessel lengthening result in the narrowing of the vessels. Pharmacological intervention with tetrodotoxin (TTX) infusion, a specific inhibitor of the fast voltage-sensitive sodium channels that underlie action potentials, also attenuated lengthening-induced vasoconstriction while neuroblockade with TTX proximal to the muscle did not influence the vasomotor response. Welsh and Segal (35) concluded that the increase in vascular resistance with muscle lengthening arises primarily from the activation of perivascular sympathetic nerves, resulting in norepinephrine release and vasoconstriction that is local in nature and does not involve afferent feedback or the cardiovascular control center. More recently, the same laboratory also revealed that muscle lengthening results in reduced conduction of vasodilation resulting from muscle length-dependent sensitivity of cell-to-cell signaling along the resistance vasculature (12). Thus it is also likely that activation of the perivascular sympathetic nerves and sensitivity of cell-to-cell signaling during knee flexion also contributed to increased resistance and decreased blood flow to the limb in the current investigation.

Muscle Length in the Knee Flexors and Knee Extensors

Limb blood flow was measured with the Doppler probe placed proximal to the bifurcation of the superficial and deep femoral artery. This placement results in quantification of blood flow to both the knee extensors as well as the knee flexors. It can thus be argued that during this cyclic passive movement the change in muscle length of the knee extensors are opposite to that of the knee flexors, thus we cannot be sure that the increase in femoral blood flow resulted from the shortening of the knee extensors or lengthening of the knee flexors. However, the knee extensors muscle volume is ~2.5 times greater than the knee flexors (3, 5, 10, 20) and the relative change in muscle length across the range of motion used in this investigation is greater in the knee extensors compared with the knee flexors (13). Taken together the knee extensors likely had a greater impact on femoral blood flow compared with the knee flexors. Furthermore, previous investigations using isolated muscle support the notion that it is in fact the shortening of a muscle that results in the increase in blood flow (18, 29).

It can also be argued that any change in blood flow in the knee extensors would be offset by a concomitant, but opposite change in blood flow to the knee flexors resulting in no change in blood flow observed in the common femoral artery. Obviously, in the current scenario, this was not the case and again we contend the knee extensors have a much larger weighting in terms of blood flow distribution compared with the knee flexors based on size and current excursion. However, the existence of at least a partial offset by these agonists likely resulted in an underestimation of the change in blood flow directed toward the knee extensor muscles as a consequence of muscle length. Measuring the blood flowing toward a single muscle or muscle group would therefore likely lead to a more dramatic result than those observed in the current study. However, this having been said, we are unaware of a vessel that does not feed an agonist/antagonist muscle pair, and blood flow can accurately be measured across a full range of motion using a Doppler ultrasound. Hence, this appears to be an unavoidable limitation of such an in vivo human experiment.

Summary

In conclusion, in the presence of constant MAP and CO, femoral blood flow was significantly altered by passive changes in knee joint angle. Specifically, during slow passive limb movement femoral blood flow decreased with knee flexion. The curvilinear relationship between femoral blood flow and increasing knee joint angle is most likely due to an increase in vessel tortuosity at the shorter muscle lengths, despite increased vessel diameter associated with reduced mechanical stretch of the quadriceps. In addition, perivascular sympathetic nerve stimulation with increasing muscle length may also contribute to the decreased blood flow with knee flexion. These data not only highlight additional factors that influence peripheral blood flow in humans at rest, but also emphasize the need for investigators, when designing research protocols, to be cognizant of the fact that joint angle, and ultimately muscle length, influence limb blood flow.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


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