Influence of venous emptying on reactive hyperemic blood flow

Zeki Bahadir
Louisiana State University and Agricultural and Mechanical College, mdzeki@hotmail.com

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INFLUENCE OF VENOUS EMPTYING ON REACTIVE HYPEREMIC BLOOD FLOW

A Thesis
Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
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in

The Department of Kinesiology

By
Zeki Bahadir
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ABSTRACT

Recent work by Tschakovsky & Hughson (Am J Physiol Heart Circ Physiol 279: H1007, 2000) indicates that venous emptying serves as a stimulus for vasodilation. This suggests the importance of recognizing the potential influence of venous volume on reactive hyperemic blood flow (RHBF) following occlusion. **Purpose:** To examine the influence of venous emptying on RHBF. **Methods:** Non-dominant forearm in-flow, venous capacitance and outflow were examined in 21 individuals [age=23±2.27y]. Forearm arterial inflow, venous capacitance, and outflow were obtained two times using strain gauge plethysmography. Forearm blood inflow was estimated at rest and following 5 min of upper arm occlusion. Forearm venous capacitance and outflow were obtained following 5 min of upper arm venous occlusion pressure at 7 mmHg below diastolic blood pressure. Prior to the second measure the arm was passively elevated for 2 minutes. Immediately before returning the arm to its original position the upper arm cuff was again inflated to 240mmHg. Subsequently, RHBF and venous measures were obtained. **Results:** Average resting in-flow was 2.84±1.22 ml/100ml/min. RHBF was significantly greater following venous emptying (Before: 18.15±3.80; After: 23.70±5.93 ml/100ml/min, p=0.0001). Venous capacitance was also greater (Before: 1.96±0.82; After: 2.94±0.82 %, p=0.0001), whereas venous outflow (Before: 37.06±10.50; After: 39.00±10.72 ml/100ml/min, p=0.17) remained unaffected after venous emptying. **Summary:** Venous emptying prior to upper arm occlusion results in a significant greater RHBF response and venous capacitance. A potential explanation for the observation is a decrease in the pressure gradient across the tissue bed and/or withdrawal of the vеноarteriolar reflex constriction.
CHAPTER 1-INTRODUCTION

Venous occlusion plethysmography is a powerful tool to study limb blood flow in humans. The technique is commonly used to study vascular function in health and disease. The basis of the technique is that a “collecting” cuff is inflated around the upper or lower limb to a pressure less than diastolic so that arterial inflow may continue whereas venous outflow is obstructed. Under this condition, the limb “swells” and the volume increases (1).

A particular interest in venous occlusion plethysmography is the study of reactive hyperemia. The classic theory behind reactive hyperemia is the autoregulatory behavior of the tissue beds. Both metabolic and myogenic influences appear to play a major role in the reactive hyperemic response.

In general, younger (2), fitter (3,4), and healthier (5,6) individuals exhibit greater reactive hyperemic blood flow (RHBF) responses following a period of occlusion. Moreover, reactive hyperemic responses are sensitive to interventions such as exercise training (3,4), diet (7, 8), and pharmacology (9,10). Recently, Tschakovsky and Hughson (11) made an interesting observation in terms of the influence of venous emptying on single conduit artery blood flow. In their study they concluded that venous emptying served as a powerful stimulus for a transient vasodilation, which substantially elevated arterial inflow. However, Tschakovsky and Hughson did not examine the influence of the emptying of the veins in the limb under study on the reactive hyperemic response following a period of occlusion. Such information is important, as the venous volume appears to have a direct impact on the rate of arterial inflow. Moreover, if reactive hyperemia is a dependent measure in interventional trials it is important to account for the venous volume as these volumes may themselves change through intervention.
The traditional method for controlling the venous volume is to position the limb above heart level to ensure proper draining. Unfortunately, there are no specific guidelines, which may help the researcher understand the manner in which the venous system can be controlled, and how this influences other vascular measures obtained. Therefore, the aim of this research study was to examine the influence of arm elevation on the RHBF response. It was hypothesized that venous emptying prior to limb occlusion would mediate a greater RHBF response. Such a finding has potential methodological and physiological consequences. In terms of the methodological importance one must consider the importance of emptying the venous system if indeed the RHBF response following venous emptying is greater than the traditional methods. In terms of the physiological consequence, the potential influence of the venous system on arterial inflow may broaden our understanding of the interplay between the two parts of the circulation, and may widen our strategies to include efforts to modify both systems.
CHAPTER 2-METHODOLOGY

Study Participants

Twenty-one individuals from the Louisiana State University student body were recruited to participate in this study. Participants were free of any symptoms of cardiovascular disease or physical/cognitive impairment. Candidates at high risk for adverse responses to exercise and/or taking any medication that may affect cardiovascular function were excluded from participating. Smokers and individuals with acute medical conditions (e.g. orthopedic injury), active infection and/or on pharmacotherapy with known vascular effects (e.g. anti-inflammatory therapy, cardiovascular medications) were also excluded. Following a comprehensive explanation of the proposed study, its benefits, inherent risks and expected commitments with regard to time, all participants signed an informed consent for exercise activities and fitness testing participation in the Kinesiology Department, which was approved by the Louisiana State University IRB.

Experimental Design

An observational prospective study was designed to examine the influence of venous emptying on reactive hyperemic blood flow. Each participant’s blood flow measurements were measured during one-visit, at three points throughout that session.

Vascular Function Assessments

Vascular function measurements were obtained in the non-dominant forearms using mercury strain gauge plethysmography at rest, following occlusion (Condition 1: C1) and following 2 minutes passive arm elevation above 90 degree of heart level (Condition 2: C2) at the session of the study. This technique is noninvasive, based on the assumption that alterations of pressure in strategically placed cuffs allow examinations of the rate of change
of limb volume thought to reflect vascular function indices, including blood inflow, vascular resistance, venous capacitance, and venous outflow (12,13). Prior to the experiment, blood pressure cuffs were positioned around each the participant’s non-dominant upper arm and wrist, and a mercury-in-silastic strain gauge placed around the forearm approximately 10 cm distal to the olecranon process (3). After 10 minutes of supine resting, the forearm vascular function measures were obtained. Immediately before the measurements, hand circulation was occluded for 1 minute by inflating the wrist cuff to 240mmHg. The resting forearm blood inflow was recorded using an upper arm venous occlusion pressure of 7mmHg below diastolic blood pressure. The forearm venous capacitance was measured after an additional 5 minutes of venous occlusion and venous outflow, following the release of pressure (4,14). Consequently, after a 5-minute resting period, the forearm reactive hyperemic blood flow was examined following 5 minutes upper arm occlusion, while inflating the cuff on the upper arm to 240mmHg. To exclude hand circulation, the wrist cuff was inflated to 240 mmHg at the 4th minute. The forearm vascular indices were determined as described above. As the final measurement, the RHBF was re-measured following venous emptying. The participant’s non-dominant arm was passively elevated 90 degrees above the heart level for two minutes, and before the forearm was repositioned, the upper cuff was inflated to 240mmHg. Subsequently, vascular measurements were recorded as described above in the regular occlusion procedure. Prior to, during and following each procedure, blood pressure and heart rate were obtained.

**Autonomic Nervous Function Assessments**

Heart Rate Variability (HRV) was used to assess autonomic nervous function. ECG electrodes were applied on the participant’s chest and attached to the Biopac MP 100. Data
were recorded continuously via software program Acqknowledge (model MP100A, Biopac Inc., Santa Barbara, CA). ECG data collected before and during the 2-minute arm elevation were analyzed for mean heart period (mean R-R interval), standard deviation. HRV analyses were made in accordance with the recommendations of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology\textsuperscript{(15,16)}.

**Data Analysis**

The plethysmographic paper speed at 5mm/sec. to record the resting forearm arterial inflow. Blood flow values were obtained by applying the classic triangular method. The first 3 pulses were taken as a baseline to draw the best-fit tangent slope to calculate the resting arterial inflow. The tangent slope increases vertically from the baseline to the top of the recording paper. Values were calculated with the formula, which is 60 seconds multiplied by the full chart range and divided by the longitudinal distance (mm), which reflects the slope between the baselines to the top of the recording paper\textsuperscript{(17)}.

The 25mm/sec. paper speed was used to record the forearm arterial inflow responses after occlusion. Since the reactive hyperemic blood flow response, after occlusion, featured two different patterns (faster and slower slopes), the triangular method was applied for each slope separately, and then the average of these was taken for the reactive hyperemic blood flow calculation. The best-fit tangent slope was determined from the first three pulses for each pattern. The blood flow values were calculated by using the formula of the paper speed (25 mm/sec), times 60 seconds, divided by 20 mm vertically to increase the slope volume’s longitudinal distance (mm)\textsuperscript{(18)}.  

5
The paper speed was set at 25 mm/sec. to record the forearm venous indices at rest and following occlusion. The forearm venous capacitance was calculated as the vertical distance (mm) reflecting the increase in the forearm-volume graph, after 5 minutes of venous filling \(^{(17)}\).

The forearm venous outflow was measured as the change in volume that was shown on the graph following venous release. The tangent was drawn on the volume line through two main points at 0.5 second and 2 seconds \(^{(17)}\).

All vascular values are presented in mL \(\cdot 100\) mL tissue\(^{-1}\). min\(^{-1}\).

**Statistical Analysis**

The statistical analysis was performed using SPSS for Windows (version 11.0). The data are presented as a mean \(\pm\) SD. T-tests were performed to examine differences between the standard condition versus the venous emptying condition. Multivariate analysis was used to determine the difference in hemodynamic responses (Heat rate, blood pressure) between resting and conditions. The Pearson correlation was used to examine the relationships between the vascular measures. The Alpha was set a priori at 0.05.
CHAPTER 3-RESULTS

Participants’ Characteristics

Twenty-one people (8 males, 13 females) between the ages of 19-26 participated in this study. Participants’ age, anthropometrics data, blood pressures, heart rate, handgrip strength are summarized in table 1.

Table 1. Participant Characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>23 ± 2.27</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>169.77 ± 9.24</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>70.65 ± 16.54</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>114 ± 8.45</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>65 ± 5.92</td>
</tr>
<tr>
<td>Heart Rate (beat/min)</td>
<td>63.95 ± 11.15</td>
</tr>
<tr>
<td>Hand Grip Strength (kg)</td>
<td>34.50 ± 13.20</td>
</tr>
<tr>
<td>Arm Circumference (cm)</td>
<td>23.95 ± 2.80</td>
</tr>
</tbody>
</table>

Values are mean ± SD. SBP: systolic blood pressure at rest, DBP: diastolic blood pressure at rest.

Hemodynamic Responses

Blood Flow Resting Measures: The average resting blood inflow for the group was 2.84 ± 1.22, average venous capacitance and venous outflow were 2.97 ± 2.80% and 34.00 ± 9.2 ml/100ml/min, respectively. No differences were noted for any of the resting measures between men and women.

Blood Flow Post Occlusion Measures: The average RHBF measures following 5 min. of upper arm occlusion was 18.15 ± 3.80 mL/100mL/min. This represents a 574 % increase, or 5.74 fold increase in blood inflow compared to the resting condition (p<0.05). The changes in blood flow following occlusion are presented in Table 2 and shown in figure 1. Average volumes for venous capacitance and outflow are also shown in table 2 and figure 2 and 3, respectively. Venous capacitance after occlusion was significantly lower compared
to rest (p<0.05). Venous outflow after occlusion was similar compared to the resting measures.

Table 2. Vascular Responses

<table>
<thead>
<tr>
<th>Condition</th>
<th>RHBF (ml/100ml/min)</th>
<th>Venous Capacitance (% volume change)</th>
<th>Venous Outflow (ml/100ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>18.15 ± 3.80</td>
<td>1.96 ± 0.82</td>
<td>37.06 ± 10.50</td>
</tr>
<tr>
<td>II</td>
<td>23.70 ± 5.93</td>
<td>2.94 ± 0.82</td>
<td>39.00 ± 10.72</td>
</tr>
</tbody>
</table>

Values are mean ± SD. RHBF: Reactive hyperemic blood flow.

Figure 1. Resting Blood Inflow and Reactive Hyperemic Blood Flow Responses.

Values are mean ± SD, Condition1 = following 5 min. occlusion, Condition2 = following 5 min occlusion preceded by 2 min arm elevation.

* = Different from Condition1.
Figure 2. Venous Capacitance.
Values are mean ± SD. Condition1 = following 5 min. occlusion, Condition2 = following 5 min occlusion preceded by 2 min arm elevation.
* = Different from Condition1.

Post Occlusion Following 2 Minutes of Arm Elevation: The average RHBF measures following 2 min. of arm elevation and subsequent 5 min. of upper arm occlusion revealed a significant difference in blood inflow compared to the traditional measure (see table 2 and figure 1). The difference was approximately 32%. Venous capacitance was also significantly higher compared to the post occlusion measures without prior arm elevation. Interestingly the average measures for venous outflow were unaffected by the maneuver. Figures 4 and 5 represent the individual changes observed for RHBF and venous capacitance between condition 1 and 2. The figures clearly indicate a consistent greater RHBF and venous capacitance following condition 2 in all but 1 subject.
Figure 3. Venous Outflow.
Values are mean ± SD. Condition1 = following 5 min. occlusion, Condition2 = following 5 min occlusion preceded by 2 min arm elevation.

Figure 4. Comparison RHBF responses.
Heart Rate / Blood Pressure / R-R Interval

Average values for heart rate and blood pressures are shown in Table 3. There were no significant changes in these measures from baseline to condition 1 and 2. In addition, the R-R intervals before (0.88 ± 0.35sec) and during arm elevation (0.9 ± 0.35sec) did not reveal significant differences. \( p=0.22 \)

Table 3. Heart rate, Blood pressure, R-R Intervals

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Condition 1</th>
<th>Condition 2</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (beat/min.)</td>
<td>62.2 ± 1.2</td>
<td>61.5 ± 10.1</td>
<td>58.8 ± 10.0</td>
<td>.579</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>113 ± 38</td>
<td>107 ± 24.8</td>
<td>111 ± 10.49</td>
<td>.549</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>5 ± 7.2</td>
<td>63 ± 7.7</td>
<td>62.5 ± 8.2</td>
<td>.533</td>
</tr>
</tbody>
</table>

Values are mean ± SD. HR: Heart rate, SBP: Systolic blood pressure, DBP: Diastolic blood pressure.
The Pearson product moment correlations for select hemodynamic measures are presented in Table 4 and Figure 6. Significant associations were found between RHBF and the venous, and blood pressure measures.

<table>
<thead>
<tr>
<th></th>
<th>RHBF (mL/100mL/min)</th>
<th>Venous capacitance (mL/100mL/min)</th>
<th>Venous outflow (mL/100mL/min)</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
<th>R-R (sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RHBF (mL/100mL/min)</td>
<td>1</td>
<td>.553**</td>
<td>.599**</td>
<td>.602**</td>
<td>.592**</td>
<td>.288</td>
</tr>
<tr>
<td>Venous capacitance (mL/100mL/min)</td>
<td>.553**</td>
<td>1</td>
<td>.733**</td>
<td>.536**</td>
<td>.617**</td>
<td>.261</td>
</tr>
<tr>
<td>Venous outflow (mL/100mL/min)</td>
<td>.599**</td>
<td>.733**</td>
<td>1</td>
<td>.400</td>
<td>.423</td>
<td>.151</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>.602**</td>
<td>.536**</td>
<td>.400</td>
<td>1</td>
<td>.919**</td>
<td>.562**</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>.592**</td>
<td>.617**</td>
<td>.423</td>
<td>.919**</td>
<td>1</td>
<td>.501</td>
</tr>
<tr>
<td>R-R (sec.)</td>
<td>.288</td>
<td>.261</td>
<td>.151</td>
<td>.562**</td>
<td>.501</td>
<td>1</td>
</tr>
</tbody>
</table>

RHBF: Reactive hyperemic blood flow following arm elevation, SBP: Systolic blood pressure following arm elevation, DBP: Diastolic blood pressure following arm elevation, R-R: heart rate intervals at rest.

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

Figure 6. Association between RHBF and Forearm Venous Outflow.
RHBF= Reactive hyperemic blood flow after occlusion following arm elevation.
CHAPTER 4- DISCUSSION

The main objective of this study was to examine the effect of adding a 2-minute period of arm elevation prior to 5 minutes of upper arm occlusion on the RHBF response. The maneuver contributes to an acute reduction in venous volume in the arm, without a significant change in heart rate, blood pressure, and measures of heart rate variability. The present study indicates that the maneuver contributes to a 30.58% greater RHBF response as compared to 5 minutes of arterial occlusion without venous emptying. This finding suggests that the initial volume in the venous system influences the reactive hyperemic blood flow responses in the forearm. Consequently, it is important to consider the influence of certain interventions, such as diet, exercise, and pharmacology, on venous volume measures, as this may provide a better understanding of the interplay between the venous and arterial systems.

The values obtained for resting and RHBF without prior arm elevation are similar to other published reports from our laboratory, and quite similar to a low cardiorespiratory fitness group previously studied. The RHBF outcome measure is generally used to differentiate individuals in terms of vascular function and, perhaps, health. The underlying assumption of the occlusion model is that the period of occlusion renders the tissue ischemic and, perhaps, hypoxic. Consequently, the tissue is reperfused upon release of the occluding pressure. The mechanisms involved in this phenomenon are many, and include metabolic waste products that accumulate during the occlusion and stimulate the opening of many precapillary sphincters in the tissue beds. In addition, myogenic and neural reflexes are also thought to play an important role in the phenomenon.
Overall the response evaluates the autoregulatory ability of the tissue to respond to a stressor.

A unique finding of the present study was that venous emptying prior to forearm occlusion resulted in significant and consistent greater RHBF responses. Although the current study was not designed to explain the change in the RHBF responses under the two conditions, several mechanisms could have contributed to the observed effect. These mechanisms include a change in the pressure gradient across the tissue bed, a change in the venoarterial reflex, or other neural reflexes.

The pressure gradient across the tissue bed may have been sufficiently altered after the venous emptying maneuver. Consequently the driving pressure across the tissue bed would have been greater, and contributed to the higher RHBF response. The finding in the present study is quite consistent with the observation by Tschakovsky and Hughson\(^\text{11}\). These investigators observed a significantly greater blood flow through a single conduit artery after arm elevation. In terms of exercise, it is perhaps important to note that a change in perfusion pressure is one of the greatest contributors to the blood flow response at the onset of exercise. The change in perfusion pressure at the onset of exercise is very much the result of the muscle pump phenomenon. In addition, the present finding is particularly interesting in light of previous work that showed that a greater perfusion gradient across the tissue beds during muscular contractions contributed to a delay in the onset of fatigue\(^\text{23}\). Although speculative it was argued that a greater perfusion gradient across the tissue bed resulted in improved metabolite washout, which consequently contributed to the delay in fatigue\(^\text{23}\).
It is interesting to consider the influence of conditions that result in venous congestion in terms of inflow characteristics. For example, Zelis and colleagues (1978) examined vascular function in patients with congestive heart failure. Their study identified that the patient group had significantly higher venous pressures, and lower resting (49%) and peak reactive hyperemic blood flow (25%) compared to healthy controls (5). Other populations who suffer from venous congestion include patients with varicose veins, and other venous insufficiencies (24, 25).

A second contributor to the observed differences between condition 1 and 2 in this study may involve a local reflex control system known as the venoarterial reflex (11). The venoarterial reflex is a local axon reflex that responds to stretching of the venous wall and contributes to arterial vasoconstriction (26). Some researchers (27, 28) suggest that a reduction of the venous pressure may result in the withdrawal of venoarterial reflex and contribute to greater arterial vasodilation (11). Nielsen et al. (28) showed that during heavy exercise, defined as 40 heel-raises per min, blood flow increased by 100%. However, no flow increase was noted during venous stasis (40 mmHg) and in areas infiltrated with lidocaine to block neural stimulus.

The present findings raise an important question regarding the RHBF response. One must ask how much the reduction in RHBF is the result of the arterial problems and how much may be secondary to alterations in venous function. Given the consistent associations between RHBF responses and measures of exercise tolerance, one should ask the question how much of exercise intolerance is secondary to problems in venous circulation. Consequently, it must be recognized that if the RHBF response is an outcome measure used to examine the influence of various treatments on the vasculature, the venous
volume and pressure must be taken in consideration. Finally, treatment plans should perhaps include the venous side, and should not solely focus on the vasodilatory capacity of the arterial circulation.

It is once again interesting to consider the strong association between the venous outflow measures and RHBF, indicating the interplay between the systems. As is shown in figure 6, this proportional relationship between these vascular beds characterizes participants’ vascular functions that who have greater arterial inflow have greater venous outflow. Hester et al. \cite{29} hypothesized that vasoactive substances may have a role in this interplay. During an increasing metabolic demand, as in exercise, the close venular-arteriolar pairing allows for diffusion of vasoactive substances from the venular blood to the arterioles. Red blood cells release adenosine triphosphate and adenosine triphosphate may stimulate the venular endothelium to release vasoactive metabolites of arachidonic acid \cite{29}. Consequently, Hester also reported that increased tissue adenosine results in upstream arteriolar dilation through venular-arteriolar diffusion \cite{30}. Injections of neoroephinephrine into precapillary vessels are the other evidence of venular controls of the arterioles, and it results in upstream arteriolar constriction at a point where the venule parallels the arteriole \cite{31}.

Future investigations should now consider to importance of venous function when examining clinical populations. Refer to Zelis \cite{5} study, effects of venous system on blood flow and vascular function, patients with congestive heart failure have limited arterial delivery, and inefficient vascular function. Given the present findings, it would appear that it is time to focus attention on strategies to improve venous function, as this may be
beneficial for patients who suffer from venous pooling and edema. Venous return and removal could be the new key factors to investigate in clinic setting.

Consistent with previous findings the present study reports that measures of venous capacitance are different before and after arm occlusion. In fact, there is approx a 34% reduction in venous capacitance after arm occlusion compared to venous capacitance at rest. The explanation for this observation is not clear, but may involve a greater number of deep tissue capillary beds that are opened following the period of occlusion. The greater number of available deep tissue capillary beds may contribute to less expansion of the arm and stretching of the strain gauge (4,19). Interestingly the present study showed that venous capacitance after the 2 min arm elevation followed by occlusion showed similar venous capacitance values as observed at rest (Figure 2). Approximately venous capacitance was 50 % greater at condition 2 than condition 1. Possible explanation could be that effects of greater RHBF responses on total forearm blood volume. Previously it has been reported that forearm blood volume decreases during arm elevation (11). Consequently, following a period of occlusion there is more room for the blood to enter the relatively empty areas, thereby yielding a greater venous capacitance.

An interesting observation is that measures of venous outflow were not different between the conditions. This lack of difference may be quite important in that it provides indirect evidence that the venous outflow is determined by the barriers within the venous system rather than the volume in the veins at the time of the measurement. Therefore this measure may in fact be independent of the volume, and provide a better reflection of the impedance within the venous system.
Obviously the interpretation of these data must be guarded in terms of the limitations of the study. Plethysmography is an indirect method to measure vascular functions and blood flow. The changes in circumferences as measured by the strain gauge do not allow individual assessment of fluid changes within specific compartments. Arterial, venous and interstitial areas cannot be assessed independently. However, high correlation ($r^2 = 0.87-0.98$) between venous occlusion plethysmography and Doppler ultrasound of the brachial artery suggest the change does reflect blood inflow $^{(1,32)}$. A second limitation of this study involves the order effect. On each occasion condition, two measurements were taken after condition 1. It is not entirely clear vascular responses are affected by prior occlusion period. Finally, the participants were all healthy college-aged students limiting the ability to extend these findings to other populations.

**Conclusion**

In conclusion, the present study suggests that venous emptying prior to arterial occlusion may contribute to a $30.58\%$ greater RHBF response as compared occlusion without prior emptying. The present finding has potential methodological and physiological consequences. In terms of the methodological importance one must consider the importance of emptying the venous system prior to measuring RHBF. Moreover, it is important to consider the possible influence of certain interventions, such as diet, exercise, and pharmacology, on venous volume measures. Knowledge of the influence of interventions on venous measures will subsequently provide a better understanding of the interplay between the venous and arterial systems.
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VITA

Zeki Bahadir is from Trabzon, Turkiye. In 1994, he received a Medical Doctorate diploma from Black Sea University, School of Medicine at Trabzon. He will receive the degree of Master of Science in kinesiology from Louisiana State University in May 2004.