OPTICAL MODALITIES FOR ASSESSING THE MICROCIRCULATION IN HYPOVOLEMIA AND ANEMIA

S.A. Bartels

It all depends on how we look at things, and not how they are in themselves.

Carl Jung

Dedicated to my parents, Niels, Maartje, and Marije

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OPTICAL MODALITIES FOR ASSESSING THE MICROCIRCULATION IN HYPOVOLEMIA AND ANEMIA

Optische modaliteiten voor de evaluatie van de microcirculatie in hypovolemie en anemie

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INTRODUCTION

THE MICROCIRCULATION – ADVANCING INSIGHTS

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INTRODUCTION

The microcirculation, consisting of the vessels with a diameter < 100 μm, is the most important system for the exchange of oxygen, carbon dioxide, and nutrients. The microcirculation plays a key role in the development of (multiple) organ failure in critically ill patients. The main aim of hemodynamic resuscitation in this patient group is to restore microcirculatory perfusion and tissue oxygenation to prevent organ hypoxia and maintain organ function [1-3]. It has been recognized that therapeutic interventions should be delivered as early as possible [3,4]. Early protocol-driven resuscitation strategies (e.g., early goal-directed therapy) targeting global hemodynamic parameters have been associated with superior clinical outcome in randomized controlled clinical trials [4,5]. However, even after interventions effectively optimizing macrocirculatory hemodynamics (e.g., cardiac filling pressure, cardiac output, blood pressure, and central or mixed venous oxygen saturation), high mortality rates still persist [6]. Rather than limiting research to macrocirculatory parameters, microcirculatory parameters should be incorporated to increase the pathophysiological knowledge underlying the disease states in these critically ill patients. Within this respect, it has been shown that improvement of macrocirculatory hemodynamics does not guarantee (sufficient) improvement of the microcirculation [2]. In this introduction we first provide a brief history of clinical monitoring of the microcirculation and we describe how microcirculatory parameters have been of great prognostic value in intensive care patients. Subsequently, we discuss the effects of resuscitation and blood transfusion on the microcirculation.

Brief history of clinical imaging of the microcirculation

The role of blood in life has intrigued people as long as mankind exists. The classical concept of Galen involved two different types of blood and lasted for many centuries. Galen presumed that one type of blood was generated in the liver and this type was responsible for growth and energy delivery throughout the body. The other type of blood originated from the heart and took care of the transport of the *spiritus vitalis*, i.e. pneumatised blood, to the body tissues. The two types of blood had their own vessel architecture. Blood was directly consumed, resulting in a non-circulatory system according to Galen [7]. For a long time, the ideas of Galen remained undebated, until Ibn al-Nafis, Michael Servetus, and William Harvey challenged the classical Galen concepts. Ibn Al-Nafis reported the concept of circulation for the first time in the 13th century, yet limited the report to the pulmonary circulation [8]. In 1553 Michael Servetus published a description of closed circuit circulation in the human body [9]. However, this hypothesis led to prosecution of Servetus and omittance of his contribution to the ideas on the circulation of the human body. In 1628 William Harvey provided a major breakthrough by describing a closed circuit circualation and suggested in *Exercitatio Anatomica de Motu Cordis et Sanguinis in Animalibus* a direct connection between the arteries and veins in the entire human body [10]. Since Harvey could not observe such a connection, he used series of relatively simple experiments to proof the concept of a closed circulation and proposed the heart as driving force in

the circulation [7]. Whereas Harvey had to speculate on the existence of very small vessels or connections, this issue was clarified by the discovery of the capillary system by Marcello Malpighi [11]. Malpighi could directly observe the existence of capillaries in animals by application of microscopy, being regarded as the final evidence. Later, Antoni van Leeuwenhoek could observe *in vivo* capillary flow for the first time [12,13].

After Van Leeuwenhoek's introduction of *in vivo* microcirculatory microscopy, the technique was long limited to semi-transparent tissue that could be transilluminated to avoid image contamination by tissue surface reflections and thereby obtain sufficient image contrast [14-16]. Later on, incident light directed at an oblique angle to the studied tissue has been applied [17]. Such a setup, however, did require very careful alignment of the light source and the microscopic lens system while it still suffered from tissue surface reflections. It was not until 1971 that Sherman et al. introduced a new method for studying the microcirculation: incident dark field illumination microscopy [18]. Dark field illumination was provided through a circular prismatic lens surrounding the objective lens which created a halo of light around and beyond the objective focal point. The method of illumination yielded "an unusual depth of field and a three-dimensional quality to the tissue observed" and permitted visualization of microcirculatory structures beneath the surface of organs as dark red blood cell columns on a bright background. The authors visualized and photographed the circulation of the cat brain, lung, kidney, liver, mesentery, and intestine successfully.

In 1987, Slaaf et al. developed an alternative way of eliminating tissue surface reflections for imaging subsurface microcirculatory networks inspired by fluorescence microscopy [19]. In fluorescence microscopy, image contrast is created by spectral separation of the reflected illumination light and the imaging light by application of an excitation and an emission filter in combination with a dichroic mirror. Similarly, Slaaf et al. proposed to separate the reflected illumination light from the imaging light by application of a polarizer and an analyzer in combination with a fifty percent reflection mirror. Due to its orthogonal orientation with respect to the polarized illumination light, the analyzer blocked reflected (undepolarized) light and allowed back scattered (depolarized) light to pass. This setting provided images of the microcirculation with sufficient contrast, similar to those obtained using dark field imaging.

Several years later, Groner et al., combined the methods developed by Sherman et al. and Slaaf et al. and added a spectral component for further optimization of image contrast. In 1999, they introduced orthogonal polarization spectral (OPS) imaging, incorporated into a hand-held, clinically-applicable device [20]. Using OPS imaging our group was the first to image the human brain microcirculation during surgery [20]. Since then, numerous studies have been undertaken in various clinical settings where cardiovascular function is at risk.

Despite the major contribution OPS imaging has made in the field of intravital microcirculatory imaging, several shortcomings are still present [21,22]. These include suboptimal imaging of capillaries due to motion-induced image blurring by movement of the OPS device, the tissue, and flow of red blood cells. The shortcomings might introduce difficulties in measuring blood flow velocities in the vessels. Thus, driven by the success of OPS imaging and its drawbacks, Goedhart et al. developed a second

generation device for clinical imaging of the microcirculation, termed sidestream dark field (SDF) imaging [23]. To date, many studies have investigated the microcirculation using OPS and SDF imaging under various pathophysiological conditions such as in surgery, emergency medicine, and intensive care medicine. Both OPS and SDF imaging have had an important clinical impact by observation of the sublingual microcirculation under various pathophysiological conditions, particularly during sepsis and shock [1-3]. Results from several medical studies have shown that alterations in the sublingual microcirculation provide more sensitive information with respect to patient outcome from sepsis and shock than conventional clinical parameters do.

In parallel to the progress in the field of microcirculatory imaging, alternative methods have been developed for the assessment of microcirculatory perfusion and oxygenation. Jobsis introduced near-infrared spectroscopy (NIRS) in 1977 [24], which assesses hemoglobin oxygen saturation in the microvasculature of tissue. In 1985, Ferrari et al. published the first clinical NIRS results [25] and afterwards many studies followed. The NIRS technique emits light at different wavelengths, corresponding to the absorption spectra of oxyhemoglobin, deoxyhemoglobin, methemoglobin, and cytochrome. The device determines the oxygen saturation in a certain amount of tissue volume, influenced by probe spacing, adiposity, and edema. Under normal physiological conditions, ~70 % of the blood volume derives from venous compartments. Clinically, NIRS was mainly used to monitor cerebral oxygenation in adults and children [26-28]. However, the results of NIRS in order to predict the effect of blood transfusion [29], identify shock [30], and critically ill patients have also been investigated [31].

Nevertheless, the role of application site and different commercially available devices currently limit the interpretation of the NIRS-derived results. Furthermore, NIRS does not take the role of blood flow into account, so interpretation of the results remains complicated. Several solutions, such as different probing depths and vascular occlusion tests, have been proposed to improve the sensitivity of NIRS in critically ill patients and healthy volunteers [32,33].

Prognostic value of the microcirculation

Microcirculatory failure is of significant prognostic value. Microcirculatory disorders before resuscitation and the persistence after resuscitation have been associated with increased risk of morbidity and mortality [1-3,34,35]. De Backer et al. found that microcirculatory alterations in non-surviving ICU patients were more severe compared to those in surviving patients [1]. This observation was later confirmed by Sakr et al. and Trzeciak et al. Furthermore, these studies showed that a lack of improvement of microcirculatory flow after resuscitation was associated with organ failure and death [2] and that non-surviving patients had significantly increased microcirculatory flow heterogeneity compared to surviving patients [3]. Doerschug et al. found that impairments in microvascular reactivity in ICU patients were related to organ failure [33]. In a later study, Trzeciak et al. demonstrated that early increases in microcirculatory perfusion during protocol-directed resuscitation were associated with reduced multiple organ failure in ICU patients [35]. Lima et al., furthermore, found that ICU

patients who consistently exhibited low microcirculatory oxygenation levels following an initial resuscitation had significantly worse organ failure in comparison with patients with normal microcirculatory oxygenation [36]. They demonstrated that oxygenation changes had no relationship with global hemodynamic variables. Besides in ICU patients, microcirculatory disorders predict mortality in patients with acute severe heart failure and cardiogenic shock [37]. Furthermore, impaired microvascular flow was associated with post-operative complications in patients with major abdominal surgery [38]. Hence, a growing body of evidence underlines the hypothesis implicating that depressed microcirculatory function can be considered as a key cause of morbidity and mortality in a wide array of clinical scenarios.

Resuscitation of the microcirculation

In their key study, Rivers et al. described an early goal-directed therapeutic protocol in which fluid resuscitation was performed untill central venous pressure was 8-12 mmHg, vasopressor agents were added to maintain the mean arterial pressure above 65 mmHg. Red blood cell transfusions and/or inotropic agents were used to increase central venous oxygen saturation to above 70 % [4]. With application of this protocol, Rivers et al significantly reduced the mortality rate in critically ill patients (31 % versus 47 % for standard therapy). Later on, Jansen et al. developed a lactate-targeted treatment protocol with the aim of preventing and treating tissue hypoxia. The protocol incorporated the vasodilators nitroglycerin and ketanserin for correction of microcirculatory derangements, the inotropes dobutamin and enoximone for correction of myocardial dysfunction, and fluids and blood transfusions for correction of hypovolemia and anemia [39]. Surprisingly, the treatment algorithm did not result in faster reduction of lactate compared to the control group, but it did significantly reduce hospital mortality. This observation demonstrates that volume replacement therapy using fluids and/or blood in combination with vasoactive agents to modulate microvascular perfusion is essential for resuscitation of severely ill patients.

Fluid resuscitation

Fluid resuscitation is probably the major therapy aimed at restoring circulating volume and consequently increasing cardiac output and arterial blood pressure in shock patients. Pottecher et al. showed that sublingual microcirculatory perfusion in severely septic and septic shock patients was significantly improved following fluid loading [40]. However, as the changes in microcirculation did not correlate to changes in macrocirculation the authors suggested that the macro- and microcirculation do not have the same dose-response to fluid loading. This was also observed by Ospina-Tascon et al. investigating the response of the macro- and microcirculation to fluid loading in the early (within 24 hours after diagnosis) or late (more that 48 hours after diagnosis) phase of septic shock [41]. The authors found that the microcirculatory parameters did increase after fluid loading in the early phase of septic shock, but not in the late phase despite significant increases in cardiac output and arterial blood pressure. In patients undergoing major abdominal surgery, Jhanji et al. compared stroke volume-guided versus central venous pressure-guided fluid therapy with respect

to their effects on microcirculatory perfusion and renal function [42]. The main result was that microvascular flow remained normal in the stroke volume-guided therapy group, but decreased in the central venous pressure-guided therapy group. Acute kidney injury was also found more frequently in the central venous pressure-guided therapy group. Hence, these studies indicate that fluid loading is an essential first step in the resuscitation of the microcirculation.

Blood transfusion

Long before William Harvey described the theory of the circulation of blood, transfusion of blood had been described on several occasions. The first known transfusion was performed on Pope Innocent VIII in 1490. The blood of three ten years old boys (who died shortly after the procedure) was supplied into the veins of the sick pope [43]. The first documented animal-to-animal transfusion attempts were performed by Richard Lower and Jean-Baptiste Dennis in 1665. In 1667, Lower and Dennis each performed first animal-to-human transfusion with sheep and lambs as donors. The next important step in transfusion medicine was taken by James Blundell, an English obstetrician, who performed his first transfusion in 1818. Between 1818 and 1829, Blundell performed 10 experiments using human blood, of which only 4 had been successful. However, until the 20th century, blood transfusion remained a risky treatment due to fatal hemolytic transfusion reactions and coagulation of donor blood. The discovery of ABO blood groups by Karl Landsteiner in 1901 and application of sodium citrate as an anticoagulant solution by Richard Lewisohn in 1915 had a major impact on transfusion medicine. The introduction of the Rhesus blood group system by Karl Landsteiner and Alexander Wiener in 1940 was again a huge step forwards. Also both World Wars heavily stimulated transfusion medicine and opened new research areas on colloid or plasma resuscitation. The past three decades were mainly focused on improving the quality of stored blood.

At present, the studies on transfusion medicine are focused on the role of the quality of the transfused blood. As the ultimate aim of RBC transfusions is to promote microcirculatory oxygen delivery, the underlying disease should also be taken into consideration as this may interfere with the efficacy of transfusion. For example, in some disease states such as sepsis, blood transfusion has been shown to be ineffective in improving the microcirculation possibly due to altered rheological, coagulation, and inflammation factors already present in the host circulation in combination with microvascular obstruction and shunting often seen in septic patients [2,4].

Only few studies have investigated the effects of RBC transfusions on the microcirculation to date [7,44-47]; only three studies have shown a beneficial effect of RBC transfusions on the microcirculation: one in adults undergoing cardiac surgery [44], one in hematological outpatients [45], and one in anemic preterm infants [46]. The contrasting results of these studies might be explained by variations in patient populations as the studies showing no effect of RBC transfusions were carried out in (septic) intensive care patients [47], in which the microcirculation is significantly impaired due to endothelial dysfunction and abnormal endogenous RBCs. These depressed microcirculatory conditions are less prevalent in surgical patients [44],

hematological outpatients [45], and preterm infants [46], possibly explaining the discrepancy between these studies.

Sakr et al. evaluated the sublingual microcirculation in 35 septic patients using orthogonal polarization spectral imaging. Measurements were performed just before RBC unit transfusion and one hour after transfusion of leukoreduced RBC units with a mean age of 24 days. Although mean arterial pressure and oxygen delivery increased following RBC transfusion, oxygen uptake and microcirculatory parameters did not. It must be noted, however, a high degree of interindividual variability was established with an increase in sublingual capillary perfusion in patients with depressed perfusion at baseline and a decrease in perfusion in patients with normal baseline perfusion [47]. Creteur et al. obtained similar results studying muscle oxygenation, oxygen consumption, and microvascular reactivity using near-infrared spectroscopy in intensive care patients receiving one leukoreduced RBC unit with a mean age of 18 days [48]. In contrast, Genzel-Boroviczény et al., in pediatric patients observed an increased functional capillary density in the skin 2 hours and 24 hours after transfusion of 9.3-14.2 mL of packed RBCs per kg body weight (age of packed RBCs unknown) [46]. In line with the study by Genzel-Boroviczény et al., our group has demonstrated an increased sublingual microcirculatory density and tissue oxygenation after transfusion of one to three RBCs units with a mean age of 18 days in cardiac surgery patients [44] and an increased sublingual and muscle tissue oxygenation after transfusion of two or three RBCs units with a mean age of 21 days in hematological outpatients [45]. In our studies we have verified the hypothesis that transfused blood is effective in improving oxygen transport to the tissue by promoting RBC delivery to the microcirculation. We have also elucidated the mechanism by which this phenomenon is accomplished: i.e., not by increasing microcirculatory flow velocity but rather by filling empty capillaries, by which the oxygen diffusion distances to the tissue cells is reduced.

CONCLUSIONS

An increasing amount of evidence exists emphasizing depressed microcirculatory function as a key cause of morbidity and mortality in a wide array of clinical scenarios. It has been shown that volume replacement therapy using fluids and/or blood in combination with vasoactive agents to modulate microvascular perfusion is essential for resuscitation of critically ill patients. However, even after interventions effectively optimizing macrocirculatory hemodynamics, high mortality rates still persist in critically ill patients. Therefore, rather than limiting therapy to macrocirculatory targets alone, microcirculatory targets should be incorporated to potentially reduce mortality rates.

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OUTLINE OF THE THESIS

The studies described in this thesis have been performed at the Department of Translational Physiology of the Academic Medical Center of the University of Amsterdam in close collaboration with the Department of Intensive Care of the Erasmus Medical Center in Rotterdam. The main goal of the collaboration between both departments is to translate physiological concepts to clinical scenarios and to introduce novel techniques into clinical practice. To this end, the Department of Translational Physiology uses controlled models for simulating clinical complications in healthy volunteers and animals. One of the key complications occurring at the Intensive Care Unit is the occult onset and progression of hypovolemia. Therefore, to study the effects of hypovolemia on the microcirculation isolated from other potentially confounding factors occurring in critically ill patients, we have developed a model in which lower body negative pressure (LBNP) is applied in healthy volunteers to induce central hypovolemia. The LBNP model allows elaborate physiological studies in subjects in which the volume status is modulated in a highly controlled environment.

As discussed in the Introduction, the microcirculation is considered to be a complex system and monitoring its status and function can provide prognostic information on patient outcome. This thesis focuses on optical modalities for the evaluation of the microcirculation in hypovolemia and anemia in subjects with a healthy microcirculation and investigates the physiological alterations consequent to hypovolemia and anemia.

Volume clamp plethysmography is used to continuously and noninvasively monitor monitor cardiac output during LBNP. To evaluate the application of volume clamp plethysmography in a wide range of cardiac output values, it has been compared to inert gas rebreathing and respired gas analysis at rest and during exercise. The results have been described in Chapter I. The study also implicates a validation for the utilization of volume clamp plethysmography during LBNP.

Several techniques might be sensitive enough to monitor alterations in the microcirculation induced by hypovolemia or anemia. First, the effect of hypovolemia on the pulse oximeter-derived peripheral perfusion index (PPI), which reflects peripheral vasomotor tone, is examined in Chapter II. Second, to explore the microcirculatory response to hypovolemia in more detail, two different microcirculatory techniques are applied in Chapter III; sidestream dark field (SDF) imaging and near-infrared spectroscopy (NIRS) in combination with a vascular occlusion test (VOT). SDF imaging enables *in vivo* visualization of the microcirculation, while NIRS measures tissue oxygen saturation. A VOT is performed to assess microvascular reactivity and to estimate tissue oxygen consumption. Third, since in Chapter III it has been shown that NIRS is a sensitive monitoring tool reflecting changes in peripheral tissue oxygenation during progressive hypovolemia, Chapter IV explores the roles of NIRS application site and probing depth on its sensitivity to hypovolemia. Finally, NIRS has been used in a clinical scenario in Chapter V; i.e., in chronic anemic hematology outpatients receiving red blood cell transfusions. Although these patients are used to low hemoglobin levels, they still require frequent red blood cell transfusions when their hemoglobin levels fall below a certain threshold. We hypothesized that anemia in these patients would be associated with low peripheral tissue oxygenation and

that this would increase after the red blood cell transfusion. This is an important issue because the studies addressing the efficacy of red blood cell transfusion at the microcirculatory level have only concerned critically ill patients and showed little or no effect on the microcirculation.

1

NONINVASIVE CARDIAC OUTPUT MONITORING DURING EXERCISE TESTING: PULSE CONTOUR ANALYSIS COMPARED TO AN INERT GAS REBREATHING METHOD AND RESPIRED GAS ANALYSIS

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ABSTRACT

Exercise testing is often used to assess cardiac function during physical exertion to obtain diagnostic information. However, this procedure is limited to measuring the electrical activity of the heart using electrocardiography and intermittent blood pressure (BP) measurements and does not involve the continuous assessment of heart functioning. In this study, we compared continuous beat-to-beat pulse contour analysis to monitor noninvasive cardiac output (CO) during exercise with inert gas rebreathing and respired gas analysis.

Nineteen healthy male volunteers were subjected to bicycle ergometry testing with increasing workloads. Cardiac output was determined noninvasively by continuous beat-to-beat pulse contour analysis (Nexfin) and by inert gas rebreathing, and estimated using the respired gas analysis method. The effects of the rebreathing maneuver on heart rate (HR), stroke volume (SV), and CO were evaluated.

The CO values derived from the Nexfin- and inert gas rebreathing methods were well correlated ($r=0.88$, $p<0.01$) and the limits of agreement were 30.3 % with a measurement bias of 0.4±1.8 L/min. Nexfin- and respired gas analysis-derived CO values correlated even better ($r=0.94$, $p<0.01$) and the limits of agreement were 21.5 % with a measurement bias of -0.70±1.6 L/min. At rest, the rebreathing maneuver increased HR by 13 bpm ($p<0.01$), SV remained unaffected ($p=0.7$), while CO increased by 1.0 L/min ($p<0.01$). Rebreathing did not affect these parameters during exercise.

We conclude that Nexfin continuous beat-to-beat pulse contour analysis is an appropriate method for noninvasive assessment of CO during exercise.

INTRODUCTION

Exercise testing is often used to assess cardiac function during physical exertion to obtain diagnostic information [1]. However, this procedure is limited to measuring electrical activity of the heart with electrocardiography, recordings of heart rate (HR), and intermittent blood pressure (BP) measurements. The method does not involve continuous assessment of mechanical heart function even though changes in stroke volume (SV) and cardiac output (CO) could provide valuable clinical information.

Clinically, CO can be measured invasively with discontinuous methods such as the direct Fick method and thermodilution, which are considered the gold standards [2-4]. These invasive methods can be cumbersome to perform and have the potential for adverse events. Several noninvasive methods for assessment of CO have been developed, such as ultrasound techniques, impedance cardiography, inert gas rebreathing, and pulse contour analysis. However, ultrasound techniques and impedance cardiography are not ideal for use during exercise. Inert gas rebreathing, on the other hand, is only considered suitable for discontinuous measurements.

As stated by De Waal et al. [5], "The ideal CO monitor should be reliable, continuous, noninvasive, operator-independent, cost-effective, and should have a fast response time (beat-to-beat)." Pulse contour analysis-derived CO assessment is considered to meet these requirements and has been incorporated into clinically applicable devices (e.g., Portapres and Finapres). However, the reliability of this method for monitoring CO has been questioned. The Finapres and its portable variant Portapres, which both employ Modelflow to derive CO measurements, were shown to be inaccurate in healthy subjects [6] and critical care patients [7], respectively. During moderate exercise, the Portapres Modelflow was shown to weakly correlate to CO_2 gas rebreathing [8]. Tam et al. compared Modelflow to rebreathing [9] and Azabji Kenfack et al. compared Modelflow to invasively-measured intra-arterial pulse pressure profiles [10] and both stated that a correction factor is required for CO values derived from the device. Bogert et al. recently evaluated a new pulse contour analysis method, the Nexfin CO-trek, designed specifically for the noninvasive assessment of finger arterial pressure [11].

The Nexfin method has been validated compared to the thermodilution method [11] incorporating values from ~3 L/min to ~9 L/min. Consequently, whether this method is capable of adequate assessment of CO during exercise, where CO rises to higher levels, remains to be established and we therefore aimed to evaluate the use of the Nexfin system for continuous noninvasive CO monitoring during exercise and an inert gas rebreathing method. Furthermore, Nexfin-derived CO values were compared to CO values estimated from whole body oxygen consumption determined by respired gas analysis, as described by Stringer et al. [12].

METHODS

A group of 19 healthy males (mean±SD age 27±5 years, height 183±6 cm, and weight 84±10 kg) was recruited for exercise testing. All participants completed a medical

history questionnaire and signed a written informed consent. The subjects refrained from intake of vasoactive medication and substances (including coffee) and intensive exercise from one day prior to the experiments. The study protocols were approved by the Medical Ethical Committee of the Academic Medical Center of the University of Amsterdam.

Nexfin blood pressure measurement

The Nexfin monitoring system (BMEYE B.V., Amsterdam, The Netherlands) is based on its predecessor, Finapres (Netherlands Organization for Applied Scientific Research, Biomedical Instrumentation (BMI-TNO)). The Finapres methodology uses the volume-clamp technique of Penáz [13] and the Physiocal calibration of Wesseling et al. [14]. Arterial volume is clamped by applying variable pressures in an inflatable cuff around the finger, which counters the pulsatile arterial pressure. An optical plethysmograph in the cuff measures arterial volume continuously. The automatic calibration system in the Nexfin determines the volume at which the artery is unloaded, i.e. when transmural pressure equals zero, assuming that the arterial wall does not interfere with the measurement. Because finger arterial pressure is different from brachial pressure in wave shape and absolute levels, waveform transformation and level corrections are applied in the Nexfin system to reconstruct brachial pressure [15]. The brachial pressure is subsequently used to determine the pulse contour derived beat-to-beat CO.

Nexfin cardiac output

Bogert et al. described the pulse contour analysis method developed for noninvasive measurement of finger arterial pressure that was incorporated into the Nexfin system [11]. The Nexfin CO-trek pulse contour method uses the pulsatile systolic area of the blood pressure curve above the diastolic pressure and between aortic valve opening and closing, as determined by upstroke and incisura. This time integral provides SV after division by arterial vascular impedance. A three element Windkessel model, based on gender, age, height, and weight of the subjects, is used to obtain impedance. Arterial impedance varies nonlinearly with pressure, which is important for the calculation of SV from the pulsatile systolic area of the blood pressure curve in the presence of changes in pressure and inherent changes in aortic stiffness and thus arterial vascular impedance. Each pulsatile systolic area provides SV. The CO is calculated as the product of SV and HR, which is determined from the interbeat interval.

Innocor inert gas rebreathing

The rebreathing device (Innocor, Innovision A/S, Odense, Denmark) measures gas concentrations in a gas mixture of enriched concentrations of oxygen (O_2) and soluble (N_2O) and insoluble (NF_6) gases from a closed rebreathing assembly [16]. During the rebreathing procedure, blood-soluble N_2O diffuses from the alveoli to the systemic circulation, and blood-insoluble NF_6 remains in the pulmonary fields. The disappearance rate in the bag volume is proportional to the pulmonary blood flow, which is assumed to be equal to the CO of the left ventricle [17]. Changes in

the composition of the mixture are measured and analyzed with a photoacoustic sensor, and the last five breaths are used by the Innocor device to calculate CO. This assumption is considered valid particularly during exercise when the pulmonary blood flow increases and pulmonary volume expands. The accuracy and precision of the inert gas rebreathing technique has been established by comparisons to the thermodilution and the Fick methods [16,18-20].

Nexfin vs. inert gas rebreathing

All of the subjects were provided with careful instructions and familiarized with the rebreathing test before the start of the protocol. Subjects were seated on a bicycle ergometer, and BP and HR were measured automatically with a sphygmomanometer placed around the left upper arm. The Nexfin finger cuff was applied on the middle phalanx of the middle finger of the right hand, which was kept at heart level using a sling to prevent the potential influence of pressure or movement artifacts during exercise. After the Nexfin signal was stabilized (defined as at least two minutes of adequate BP traces without oscillations), the baseline rebreathing test was performed according to the instructions of the manufacturer. After baseline measurements were successfully collected, the participants started to cycle with an increasing workload of 25 Watts/min until their HR reached 150 bpm. Another rebreathing test was subsequently performed. The rebreathing procedure consisted of five to seven breaths at a breath rate of 20/min and the last five breaths (i.e., 15 seconds) were used for derivation of CO. Nexfin provided beat-to-beat values for CO, which were averaged over the same period as the last five breaths of the rebreathing procedure (i.e., 15 seconds, regardless of the HR).

Nexfin vs. repired gas analysis

A subset of the original subject population (n=9) participated in an additional exercise protocol similar to the protocol used to compare the Nexfin- and Innorcor-derived CO measurements. In this study, the exercise rate was increased until exhaustion. During this protocol, volume was measured with a bi-directional digital volume transducer (DVT; dead space 30 mL), which was attached to a face mask (Hans Rudolph Inc, Kansas City, MO; dead space 40 mL). Oxygen uptake (VO_2) and carbon dioxide production (CO_2) were measured using the Oxycon Pro (Viasys Healthcare, Hoechberg, Germany). VO_2 and CO_2 were averaged over 30 seconds. VO_2max was defined as the average VO_2 in the last 30 seconds of the experiment (i.e., at exhaustion), and the $\%VO_2$max was calculated as $VO_2/VO_2max \cdot 100$. According to Stringer et al. [12], CO can be estimated from VO_2 and $\%VO_2$max using the following equation: estimated $CO=0.1 \cdot VO_2/(5.721+(0.1047 \cdot \%VO_2max))$. Figure 3 shows the Nexfin- and respired gas analysis-derived cardiac output measured at different workloads.

Data analysis

Nexfin-derived CO values were compared to inert gas rebreathing- and respired gas analysis-derived CO values using Spearman correlation and linear regression analysis.

Agreement between the techniques was assessed using Bland-Altman analysis [21] and Critchley and Critchley analysis [22]. Bland-Altman analysis generates a plot of the difference between the CO values measured with two techniques versus the average value measured by the techniques and provides a measurement bias expressed as the mean±SD [21]. The Critchley and Critchley analysis calculates the limits of agreement by dividing 2xSD of the CO measurement bias by the overall measured average CO. Critchley and Critchley have proposed that the limits of agreement should be ≤30 % to represent proper agreement between two techniques [22], while Peyton and Chong accept a cut-off value of 45 % [23].

To determine the effects of the rebreathing test on hemodynamic parameters, Nexfin-derived HR, SV, and CO were measured and averaged for 15 seconds before the rebreathing test and for 15 seconds during the test. The data are expressed as mean±SD. The statistical significance of the difference in values obtained before and during the rebreathing test was determined using the Wilcoxon signed rank test. Values of $p<0.05$ were considered statistically significant.

RESULTS

Nexfin vs. inert gas rebreathing

Figure 1A depicts the Nexfin- versus inert gas rebreathing-derived CO values, which were highly correlated (Spearman $r=0.88$, $p<0.01$). In Figure 1B, a Bland-Altman plot shows a measurement bias of 0.43±1.8 L/min between the Nexfin and inert gas rebreathing. The mean±SD CO of the data set (i.e., rest and exercise, and Nexfin and inert gas rebreathing measurements) was 11.9±4.6 L/min. The overall limits of agreement were (2x1.8/11.9) 30.3 %. The limits of agreement at rest were somewhat higher (34 %) than during exercise (26 %).

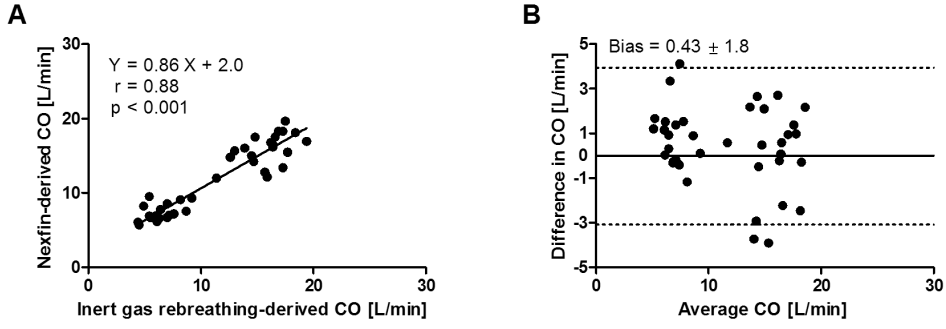

Figure 1. Panel A shows the values of Nexfin- versus inert gas rebreathing-derived cardiac output (CO). Panel B shows a Bland-Altman plot depicting the difference between the CO values measured by the two techniques (i.e., Nexfin-derived CO values minus inert gas rebreathing-derived CO values) versus the average value measured by the techniques.

Nexfin vs. respired gas analysis

Figure 2A depicts the Nexfin- versus respired gas analysis-derived CO values, which were highly correlated (Spearman r=0.94, p<0.01). In Figure 2B, a Bland-Altman plot shows a measurement bias of -0.70±1.6 L/min between Nexfin and respired gas analysis. The mean±SD CO of the data set (i.e., Nexfin and respired gas analysis measurements) was 14.9±4.5 L/min. The overall limits of agreement were (2x1.6/14.9) 21.5 %. Figure 3 shows the Nexfin- and respired gas analysis-derived cardiac output measured at different workloads.

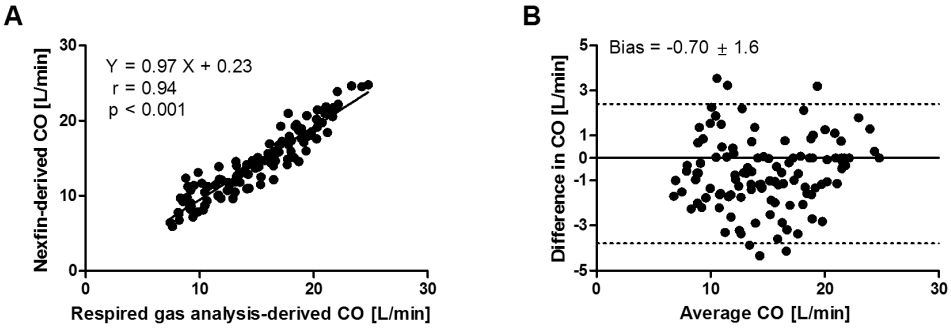

Figure 2. Panel A shows the values of Nexfin- versus respired gas analysis-derived cardiac output (CO). Panel B shows a Bland-Altman plot depicting the difference between the CO values measured by the two techniques (i.e., Nexfin-derived CO values minus respired gas analysis-derived CO values) versus the average value measured by the techniques.

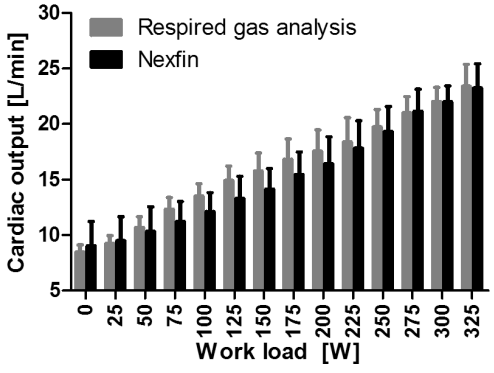

Figure 3. Nexfin- and respired gas analysis-derived cardiac output measured at different workloads. A two-way analysis of variance showed that p>0.05 at all work load levels, indicating no significant differences between Nexfin and respired gas analysis-derived measurements.

Effects of the inert gas rebreathing method on HR, SV, and CO

In table 1, the Nexfin-derived HR, SV, and CO measurements before and during the Innocor rebreathing test shows the effects of the rebreathing test on HR, SV, and CO at rest and during exercise. At rest, the CO increased from 6.6±1.3 before the rebreathing test to 7.6±1.5 L/min ($p<0.01$) during the test. This is the result of an increase in HR from 83±11 to 96±13 bpm ($p<0.01$). SV did not change significantly during rebreathing ($p=0.73$). The rebreathing test did not affect CO, HR, or SV during exercise.

Table 1. The values of Nexfin-derived heart rate, stroke volume, and cardiac output during rest and exercise and before and during the Innocor inert gas rebreathing test.

		Rest			Exercise		
		Before rebreathing	During rebreathing	p-value	Before rebreathing	During rebreathing	p-value
Heart rate	bpm	83±11	96±13	< 0.01	150±6	151±5	0.30
Stroke volume	mL	80±9	80±13	0.73	106±13	107±14	0.89
Cardiac output	L/min	6.6±1.3	7.6±1.5	< 0.01	15.9±1.9	16.1±2.0	0.61

Table 2. The cardio-respiratory performance of a cohort of 9 subjects performing an exercise test with increasing work load for comparison of respired gas analysis-derived and Nexfin-derived cardiac output values.

	Mean±SD
Maximum work rate	286±40 W
Maximum oxygen uptake	3.2±0.5 L/min
Maximum breathing frequency	39±3/min
Maximum heart rate	182±9 bpm
Maximum cardiac output	20.0±3 L/min
Maximum respiratory exchange ratio	1.3±0.1
Maximum VO_2/kg	41±6 mL/min/kg
Maximum oxygen pulse	19±4 mL/bpm

DISCUSSION

The aim of this study was to compare the use of the Nexfin system for continuous noninvasive CO monitoring during exercise compared with inert gas rebreathing and respired gas analysis. Nexfin- and inert gas rebreathing-derived CO values were well correlated and the limits of agreement marginally meet the Critchley and Critchley criterion (i.e., 30.3 % versus the threshold of ≤30 %). Nexfin- and respired gas analysis-derived CO values were better correlated, and the limits of agreement easily met the Critchley and Critchley criterion (i.e., 21.5 %). We demonstrated that the

rebreathing method significantly increased HR and CO and that CO measurements at complete rest are therefore not possible using this method.

Previous studies on the application of pulse contour analysis for CO assessment during exercise have suggested that a calibration factor is needed [9,10]. In contrast, Sugawara et al. described good correlation between Portapres Modelflow and Doppler echocardiography during exercise [24]. We found that Nexfin-derived CO values correlated well with inert gas rebreathing- and respired gas analysis-derived CO values with an acceptable measurement bias of ≤0.7 L/min. The limits of agreement for Nexfin and inert gas rebreathing were higher at rest (34 %) than during exercise (26 %). Hence, the limits of agreement met the Critchley and Critchley criterion during exercise, but not at rest. The proposed 45 % benchmark from Peyton and Chong [23] was easily met during both rest and exercise. The limits of agreement for Nexfin and respired gas analysis [12] were far below the Critchley and Critchley criterion (i.e., 21.5 %). Collectively, these results show that Nexfin can be used to measure CO over a large range of CO values (table 2).

The Nexfin determinations of CO were an average of ~0.5 L/min higher than the Innocor measurements, especially at rest. It has been suggested that rebreathing tests are more accurate when there is a high CO compared to a low CO and that the accuracy of this method could be improved as a result of exercise [25]. The physiological explanation regarding the improved accuracy of the rebreathing method during exercise could be that increased pulmonary volume and blood flow and decreased shunting leads to an improved mixing of gases because incomplete mixing has been shown to result in an underestimation of CO [18,19]. However, a study by Jarvis et al. showed an underestimation of CO by rebreathing during exercise and suggested that an increased velocity of blood flow during exercise shortens the recirculation time of the rebreathing gas mixture [26]. This discrepancy might be due to the use of different rebreathing gases (C_2H_2 instead of N_2O and NF_6) or variations in protocols. Because there are a variety of devices and gas mixture compositions used in the rebreathing method, the results from different studies should be compared with caution.

In this study, we chose an inert gas rebreathing method for evaluation of the Nexfin method for assessment of CO because this technique measures CO noninvasively. However, rebreathing is a discontinuous method that may constitute an extra load to perform during exercise, especially for patients with heart failure, and has been shown to significantly increase HR and CO [17,27]. This we have also shown here. Hence, although this technique enables the noninvasive quantitative assessment of CO during exercise, it might not be the method of choice in every clinical scenario. In contrast, pulse contour analysis provides a reliable, continuous, noninvasive, operator-independent, and beat-to-beat responsive method for assessment of CO in healthy subjects and patients who have undergone cardiac surgery [11]. In addition to inert gas rebreathing, we compared the Nexfin-derived CO values to estimated CO values derived from respired gas analysis. The respired gas analysis method has been introduced by Stringer et al. and was compared to the direct Fick method [12]. The authors demonstrated that CO can be accurately estimated from VO_2 and VO_2max during exercise in normal subjects and patients with heart failure.

We acknowledge several shortcomings in this study. First, we did not include one of the gold standard methods for CO assessment in our protocol, such as thermodilution or the Fick method. However, the Nexfin, the inert gas rebreathing method, and the respired gas analysis method have all been compared to these gold standards [11,12,25] and applying thermodilution or the Fick method during exercise could introduce adverse events due to the invasive nature of these techniques. In addition, the rebreathing test, the Fick method, and the thermodilution method all calculate an average CO over a certain period of time, assuming a steady state condition. However, even successive repetition of the thermodilution method under stable hemodynamic conditions result in alterations of CO by >1.0 L/min [28], indicating that CO can vary substantially during a short period of time. The safety and validity of thermodilution were questioned in a study by Williams et al. [29]. In addition, agreement between the Fick method and the thermodilution method remains a debate [30,31]. Other noninvasive methods for continuous CO assessment, such as ultrasound techniques and impedance cardiography, are not ideal for use during exercise. Second, rebreathing measurements were performed at a fixed breathing rate of 20/min both at rest and during exercise. Because the Nexfin provides beat-to-beat measurements of CO, there were more Nexfin measurements averaged over the rebreathing interval (i.e., 15 s, regardless of HR) during exercise compared to the measurements at rest. Third, although we compared the continuous Nexfin measurements of CO to the discontinuous inert gas rebreathing measurements, we did not perform repeated rebreathing measurements as suggested by Peyton et al. [20], because rebreathing tests can be quite exhausting and require a specific breathing rate and volume for accurate measurements. Overall, we found that Nexfin- and inert gas rebreathing-derived CO measurements were well correlated over a large range of CO values and, using the Nexfin system, we demonstrated that the rebreathing method significantly affected HR and CO at rest.

CONCLUSIONS

Nexfin continuous beat-to-beat pulse contour analysis is an appropriate method for noninvasive CO assessment during exercise and the rebreathing method significantly affected HR and CO at rest.

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II

PERIPHERAL PERFUSION INDEX AS AN EARLY PREDICTOR FOR CENTRAL HYPOVOLEMIA

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ABSTRACT

To investigate the ability of the Peripheral Perfusion Index (PPI) to reflect early peripheral vasoconstriction during progressive hypovolemia, we applied lower body negative pressure (LBNP) in twenty-five healthy male volunteers until onset of cardiovascular collapse. and we compared the ability of volume clamp plethysmography and PPI to induced central hypvolemia.

Twenty-five healthy volunteers were subjected to a stepwise LBNP protocol from 0 to -20, -40, and -60 mmHg (5 min per step). Throughout the procedure, stroke volume (SV), heart rate (HR), blood pressure (BP), systemic vascular resistance (SVR), and cardiac output (CO) were recorded using volume clamp finger plethysmography (Nexfin). Assessment of the PPI was done by pulse oximetry (Masimo SET).

During the first LBNP step (-20 mmHg), SV decreased (10 %, $p<0.001$), while HR remained unaltered. In contrast, SVR increased (10 %, $p<0.001$). PPI decreased significantly with 47 % ($p<0.001$). During further progression of LBNP, SV decreased and HR increased proportionally to the applied negative pressure. SVR and PPI remained around the LBNP=-20 mmHg level throughout the rest of the protocol. As a result of adequately functioning compensatory mechanisms, BP and CO did not change significantly throughout the experiment.

We conclude that LBNP results in a decrease in SV and an increase in HR and SVR. The latter two can be successfully monitored using the applied pulse oximeter. PPI can be used as a complementary hemodynamic monitoring technique for the early detection of central hypovolemia.

INTRODUCTION

Disturbance of the peripheral perfusion can be an early predictor of tissue hypoperfusion and as such a warning signal of ongoing circulatory shock in critically ill patients [1-2]. Till date, detection of hypovolemia in the early phase remains of prime importance, but is complicated to monitor due to physiological compensatory responses , such as increased heart rate (HR) and systemic vascular resistance (SVR), induced by hypovolemia. Vasoconstriction in the peripheral tissues redistributes the blood flow to more central compartments [3-5] and limits the usefulness of mean arterial pressure (MAP) and cardiac output (CO) as indicators for mild hypovolemia during early stage of shock [6]. When conventional hypovolemic signs occur, circulating blood volume is already ≥30 per cent diminished. At this point, most of the CO is used to maintain perfusion of vital organs, resulting in abnormal tissue perfusion of peripheral tissues and, eventually, hypovolemic shock [7]. Therefore it is important to recognize hemodynamic instability before this point. Monitoring of the early compensatory peripheral mechanisms might better indicate the onset of hypovolemic or low flow shock in the acute stage of the disease [4].

The peripheral perfusion index (PPI), derived from the photo-electric plethysmographic signal of the pulse oximeter, has been established as a very useful noninvasive method for the assessment of peripheral vasomotor tone in healthy volunteers and critically ill patients [2,8]. Because a pulse oximeter is universally available in the operating room, emergency room, and intensive care unit, it could potentially be used for the detection of peripheral hypoperfusion in the early response to ongoing hypovolemia in these settings. However, the sensitivity of this modality to mild changes in volume status has never been investigated in a controlled model of hypovolemia.

In the present study we therefore aimed to test the hypothesis that the PPI would decrease (reflecting peripheral vasoconstriction) early in the response to progressive hypovolemia. To this end, we employed a model of controlled central hypovolemia in healthy volunteers: lower body negative pressure (LBNP). During application of LBNP, the circulating volume is redistributed from the upper to the lower body creating central hypovolemia [9], which allows studying the cardiovascular responses to hypovolemia under controlled conditions.

METHODS

Subjects

This study was conducted in a research laboratory at a university affiliated teaching hospital. We recruited twenty-five healthy volunteers with no history of cardiac events nor receiving any vasoactive medication. All participants completed a medical history and underwent an exercise electrocardiogram. To minimize potential confounding factors [10], we recruited a homogenous group of male volunteers in terms of physical fitness and age. The volunteers were instructed not to consume caffeine-containing drinks or practice intensive exercise ≤12 hours prior to the experiments. The study protocol was approved by the Medical Ethics Committee of the Academic Medical

Center of the University of Amsterdam, and written informed consent was obtained from all subjects.

Measurements

Subjects were placed with their lower body in an air-tight chamber with a seal applied at the level of the iliac crests. The negative pressure within the chamber could be manually adjusted using a variable vacuum source. Baseline recordings were made after a 10-min stabilization period prior to LBNP application. Then, LBNP was applied progressively at a 5 minute stepwise fashion from 0 to -20, -40, and -60 mmHg. The LBNP protocol could be immediately terminated when the subject developed presyncope, which was defined as a drop in systolic blood pressure of >15 mmHg from baseline, or experienced symptoms of impending syncope such as dizziness, light-headedness, nausea, or visual disturbances [11].

Hemodynamic parameters

Hemodynamic parameters, including MAP, CO, stroke volume (SV), HR, and SVR, were continuously and non-invasively measured using volume clamp finger plethysmography (Nexfin, BMEYE, Amsterdam, The Netherlands) with the cuff placed around the left index finger. The Nexfin device is extensively described elsewhere and has shown to provide reliable measurements of these hemodynamic parameters [12-13]. In brief, this method applies variable pressure in an inflatable cuff around the finger, countering the pulsatile arterial pressure. An optical plethysmograph placed in this cuff measures arterial volume and a calibration system determines the volume at which the artery is unloaded (i.e. when transmural pressure equals zero and no interference of the arterial wall occurs). Since brachial and finger arterial pressure are physiologically different, waveform transformation and correction are applied in order to reconstruct brachial arterial pressure. The study of Bogert et al. demonstrated that Nexfin-derived CO correlated well to thermodilution-derived CO. Systemic vascular resistance is calculated as SVR = ([MAP – central venous pressure] • 80/CO). In the Nexfin device, central venous pressure is set at 5 mmHg, which might lead to minor inaccuracies, especially at very low MAP. However, as we only monitored hemodynamic under compensated conditions, MAP remained sufficiently high and SVR could be estimated reliably [14].

Peripheral Perfusion Index (PPI)

The PPI (Masimo SET® pulse oximetry Perfusion Index, Radical 7, Basingstoke, Hants, UK) provides a noninvasive parameter reflecting changes in peripheral vasomotor tone [15-16]. It is derived from the photo-electric plethysmographic signal of the pulse oximeter and has been used as a noninvasive measurement of peripheral perfusion in healthy volunteers and critically ill patients [2,8]. The technique is based on two light sources that emit light at wavelengths of 660 and 940 nm through the cutaneous vascular bed of the distal side of the index finger. As other tissues, such as connective tissue, bone, and venous blood, also absorb light, the pulse oximeter distinguishes the pulsatile component of arterial blood from the nonpulsatile

component of venous and capillary blood, and other tissues. Using a two-wavelength system the pulsatile component is used to calculate the arterial oxygen saturation. The PPI is calculated as the ratio between the pulsatile component (arterial compartment) and the nonpulsatile component (venous and capillary blood, and other tissues) of the light reaching the detector of the pulse oximeter. This ratio is independent of the hemoglobin oxygen saturation. Because a change in peripheral vasomotor tone primarily causes a proportional change in the pulsatile component of the signal, the PPI changes accordingly. As a result, the PPI value reflects changes in peripheral vasomotor tone.

Statistics

Unless otherwise specified, results are presented as median [25 % - 75 %]. Comparative analysis of data sets was performed using (nonparametric) repeated measurements one-way analysis of variance tests. Data were plotted and analyzed using GraphPad Prism software (GraphPad Prism Software, San Diego, CA). $P<0.05$ was regarded statistically significant.

RESULTS

Of the 25 male subjects (mean ± SD age 23 ± 6 yrs; weight 82 ± 2 kg; and height 182 ± 1 cm), 25 completed the LBNP=-20 and -40 mmHg steps and 24 completed the LBNP=-60 mmHg step.

Global hemodynamic parameters and PPI are presented in Table 1 and Figure 1A. At onset of mild central hypovolemia (i.e., from baseline to LBNP=-20 mmHg), SV decreased from 113 [107-126] mL to 101 [95-112] mL (i.e., -11 %), HR remained unaltered (59 [53-69] bpm to 62 [59-68] bpm ($p>0.05$), while SVR significantly increased from 1023 [972-1140] to 1141 [1057-1267] AU (+12 %; $p<0.001$). Progressive mild hypovolemia (i.e. LBNP≤-40 mmHg) resulted in a progressive decrease of SV (≤20 % of baseline; $p<0.001$) and increase of HR (≥ +17 %; $p<0.001$). SVR remained elevated around the same level. MAP and CO were maintained around baseline levels throughout the experiment.

Table 1. Descriptive analysis (median [25 % -75 %]) of heart rate (HR), stroke volume (SV), cardiac output (CO), mean arterial pressure (MAP), systemic vascular resistance (SVR), and peripheral perfusion index (PPI) during progressive application of lower body negative pressure (LBNP) from 0 mmHg to -60 mmHg. ‡ $p<0.001$ vs. LBNP = 0 mmHg, * $p<0.001$ vs. previous time point.

LBNP level	0 mmHg	-20 mmHg	-40 mmHg	-60 mmHg
HR (bpm)	59 [53-69]	62 [59-68]	69‡* [66-81]	84‡* [78-95]
SV (mL)	113 [107-126]	101‡* [95-112]	89‡* [83-98]	74‡* [66-83]
CO (L/min)	6.7 [6.2-7.6]	6.2 [5.9-7.1]	6.0 [5.8-7.0]	6.0 [5.7-6.9]
MAP	91 [84-97]	92 [88-99]	91 [85-95]	87 [83-94]
SVR (AU)	1023 [972-1140]	1141‡* 1057-1267]	1152‡ [1035-1267]	1120‡ [1016-1278]
PPI (%)	2.3 [1.6-3.6]	1.2‡* [0.8-1.8]	1.2‡ [0.9-1.9]	1.3‡ [0.9-1.7]

Similar to SVR, PPI immediately decreased at the onset of LBNP from 2.3 [1.6-3.6] % to 1.2 [0.8-1.8] % ($p<0.001$) at LBNP=-20 mmHg and remained around this level during the rest of the protocol. Comparing relative changes, PPI demonstrated to be highly sensitive with a 47 % decrease at LBNP=-20 mmHg compared to the 11 % decrease in SV, the 5 % increase in HR, and the 12 % increase in SVR (Figure 1A). Changes in PPI showed no significant differences after the first decrease at LBNP=-20 mmHg. Interestingly, this pattern is similar to the SVR, albeit that the magnitude of change was significantly larger for the PPI.

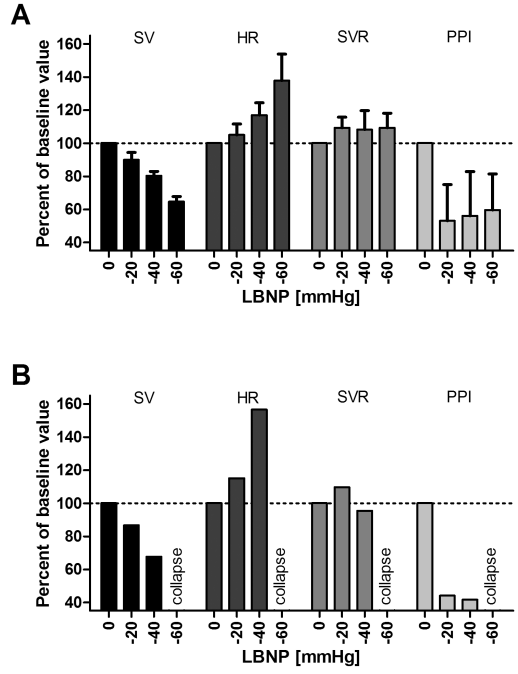

Figure 1. Stroke volume (SV), heart rate (HR), systemic vascular resistance (SVR), and peripheral perfusion index (PPI) plotted as percentage of baseline during progressive application of lower body negative pressure (LBNP) from 0 mmHg to -60 mmHg **(A)** and the same parameters in the person with impending cardiovascular collapse at LBNP=-40 mmHg **(B)**.

One subject did not complete the study due to impending cardiovascular collapse. Because this persons' response to increasing negative pressure was markedly different from the other participants, we show the time course of the relative changes in SV, HR, SVR, and PPI in Figure 1B. In the non-collapsing subjects at LBNP=-40 mmHg, the median SV was 89 mL, HR was 69 bpm, SVR was 1152 AU, and PPI was 1.2 %, while in the collapsing subject the SV was 86 mL, HR was 83 bpm, SVR was 1014 AU, and PPI was 0.5 %. Hence, especially HR and PPI differed markedly between the collapsing subject and the non-collapsing subjects. This combination of very high HR and very low PPI at LBNP=-40 mmHg was only found in the subject that collapsed.

DISCUSSION

The aim of the present study was test the hypothesis that progressive central hypovolemia created by application of LBNP would result in a decreased PPI, reflecting peripheral vasoconstriction in response to hypovolemia. We found that PPI was highly sensitive to mild hypovolemia at LBNP=-20 mmHg with a 47 % decrease compared to the 11 % decrease in SV, the (non-significant) 5 % increase in HR, and the 12 % increase in SVR, but was unresponsive during further reduction of the central circulating volume at higher levels of LBNP. Furthermore, we have shown that, in contrast to the SVR and PPI, HR was unresponsive to the first step of LBNP, but did increase progressively at higher levels of LBNP. During the entire protocol, CO and MAP were maintained around baseline levels which indicated adequate compensation of the reduced central hypovolemia. As such we have demonstrated a biphasic response to progressive hypovolemia in which first the peripheral perfusion is decreased (as reflected by increased SVR and decreased PPI) and subsequently the HR is increased. This illustrates that PPI might be a valuable parameter for detecting the onset of hypovolemia and, under conditions of significantly reduced PPI, HR might be used to monitor further progression of hypovolemia (although HR might be affected by other factors also associated with critical illness). Both parameters can be obtained from a single pulse oximeter.

The early detection of hypovolemia is of key importance in the management of critically ill patients. However, physiological compensatory factors, such as increased HR and SVR, limit the use MAP and CO as indicators for hypovolemia [6]. When CO and MAP start to fall, circulating blood volume is already ≥30 % reduced [7]. Therefore, recognizing hemodynamic instability before this point is key in the prevention of hypotension and hypoperfusion-induced (multiple) organ failure in these patients. To this end, monitoring the early compensatory peripheral mechanisms might provide better parameters indicating the onset of hypovolemia [4]. As shown here and in previous studies, the pulse oximeter-derived PPI might be a very useful and noninvasive method for the detection in changes in peripheral vasomotor tone in response to volume status alterations [2,8].

A possible explanation for the rapid response of PPI at onset of hypovolemia and its non-responsive behavior during further progression of hypovolemia could be that of limited vasoconstrictor reserve [17]. The concept of vasoconstrictor reserve is paralleling the chronotropic reserve, i.e. the major determinant of residual CO capacity. During normal conditions, a rise in HR and a functional sympathetic response during orthostatic stress is aimed at maintaining CO within normal physiological limits [18]. However, the degree of sympathetic reserve available for vasoconstriction is finite and may vary among subjects/patients, it might be one of the determinants underlying the individual variability in orthostatic tolerance [19].

Previous studies [20-21] suggest that lack of vagal tone results in reduced or total loss of vasoconstriction and occur at the onset of cardiovascular collapse. Hemodynamic instability leading to this collapse has been associated with reduced acute sympathetic baroreflex response [22-23] and abrupt sympathetic neural withdrawal [21]. Cardiovascular collapse becomes imminent when cardiac chronotropic

and peripheral vasoconstrictive mechanisms can no longer adequately compensate for progressive hypovolemia. This is supported by previous studies demonstrating the phenomenon of paradoxical vasodilation in patients with depressed baroreceptor unloading [24] and congestive heart failure [25] during mild central hypovolemia (i.e. LBNP = -20 mmHg) induced by LBNP.

The PPI has been shown to be an early indicator of acute changes in peripheral vasomotor tone in response to changes in central blood volume. The inclusion of PPI in pulse oximetry is a recent advance in clinical monitoring and makes it easy applicable at the bedside. This noninvasively assessed PPI has been found to correlate with hypoperfusion in critically ill patients and can be used to predict the severity of illness [1,2,15,26]. We therefore suggest that, in combination with HR, PPI can be used as a complementary hemodynamic monitoring technique for the early detection of hemodynamic instability in trauma and intensive care patients. It should be noted though, that PPI remains unresponsive during further progression of hypovolemia and cannot differentiate between progressive hypovolemia and mild hypovolemia, which might be of vital importance for follow up and resuscitation.

This study has some limitations that should be acknowledged. First, although volume redistributions with LBNP are similar to those that occur during hemorrhage, we did not induce severe hypovolemia or hypovolemic shock. Instead, we were interested in the ability of the PPI to detect early peripheral vasoconstrictor responses in the compensated phase of hypovolemia. This, we clearly showed, as changes in PPI indeed occurred immediately at onset of the LBNP-induced volume shift. Second, we did not determine systemic hemodynamic parameters using invasive methods such as arterial or venous catheters, but we have used the noninvasive Nexfin system. This system, as well as its predecessors, have been extensively validated and have been shown to provide reliable measurements of cardiovascular parameters [12]. Third, because the PPI is derived from the plethysmographic signal, it has been questioned whether, in case of peripheral vasoconstriction, the pulse waveform is sufficient to register acute hemodynamic perfusion changes [27]. However, we found no indications during our protocol that the detected pulse waveform was of insufficient amplitude. Furthermore, it has previously been shown that PPI is a reliable technique for monitoring peripheral perfusion during systemic hemodynamic changes in critically ill patients [2,8] and during major surgery [28].

CONCLUSIONS

The pulse oximeter-derived PPI is a sensitive indicator for the early detection of hemodynamic compensation in response to LBNP-induced central hypovolemia. As such, PPI can be used as a complementary hemodynamic monitoring technique for the early recognition of hemodynamic instability in intensive care patients.

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III

THE MICROCIRCULATORY RESPONSE TO COMPENSATED HYPOVOLEMIA IN A LOWER BODY NEGATIVE PRESSURE MODEL AS STUDIED USING SDF IMAGING AND NIRS

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ABSTRACT

The objective of the present study was to test the hypothesis that controlled, adequately compensated, central hypovolemia in subjects with intact autoregulation would be associated with decreased peripheral microcirculatory diffusion and convection properties and, consequently, decreased tissue oxygen carrying capacity and tissue oxygenation. Furthermore, we evaluated the impact of hypovolemia-induced microcirculatory alterations on resting tissue oxygen consumption. To this end, 24 subjects were subjected to a progressive lower body negative pressure (LBNP) protocol of which 14 reached the end of the protocol. At baseline and at LBNP=-60 mmHg, sidestream dark field (SDF) images of the sublingual microcirculation were acquired to measure microvascular density and perfusion; thenar and forearm tissue hemoglobin content (THI) and tissue oxygenation (StO2) were recorded using near-infrared spectroscopy (NIRS); and a vascular occlusion test (VOT) was performed to assess resting tissue oxygen consumption rate. SDF images were analyzed for total vessel density (TVD), perfused vessel density (PVD), the microvascular flow index (MFI), and flow heterogeneity (MFIhetero). We found that application of LBNP resulted in: 1) a significantly decreased microvascular density (PVD) and perfusion (MFI and MFIhetero); 2) a significantly decreased THI and StO2; and 3) an unaltered resting tissue oxygen consumption rate. In conclusion, using SDF imaging in combination with NIRS we showed that controlled, adequately compensated, central hypovolemia in subjects with intact autoregulation is associated with decreased microcirculatory diffusion (PVD) and convection (MFI and MFIhetero) properties and, consequently, decreased tissue oxygen carrying capacity (THI) and tissue oxygenation (StO2). Furthermore, using a VOT we found that resting tissue oxygen consumption was maintained under conditions of adequately compensated central hypovolemia.

INTRODUCTION

Hypovolemia is a common clinical complication occurring in operating rooms, emergency rooms, and intensive care units [1-3]. The hypovolemia-associated reduction in stroke volume (SV) is physiologically countered by compensatory mechanisms such as increased heart rate (HR) and peripheral (micro)vascular tone to prevent the consequent decrease in blood pressure and organ perfusion [4-6]. The peripheral microcirculation therefore plays a critical role in the response to hypovolemia, which has been demonstrated by Ward, Soller, and colleagues [7-9]. However, the exact response of the microcirculation with respect to (down)regulating microvascular density and perfusion and the consequent effects on peripheral tissue oxygenation and oxygen consumption for compensation of hypovolemia remains elusive.

To evaluate the microcirculation under hypovolemic conditions, Ward et al., recently performed sidestream dark field (SDF) imaging during lower body negative pressure (LBNP) in healthy subjects [7]. LBNP reduces central blood volume, as demonstrated by several studies [10,11]. In agreement with earlier studies [8,9], application of LBNP resulted in a significant shift in blood volume from the upper to the lower body that was accompanied by a reduction in SV and allowed to study the effects of controlled central hypovolemia on the microcirculation. The authors found that microvascular density was significantly reduced by the application of LBNP [7]. However, the authors only reported on changes in microvascular density and did not describe the potential changes in microvascular flow. Therefore, although the study provided useful insight in the diffusion-related changes (microvascular density) in the microcirculation in response to hypovolemia, it lacked insight in the convection-related changes (microvascular perfusion). This is, however, a significant variable since downregulation of the microcirculation for maintenance of blood pressure potentially relies on two pathways; i.e., reducing microvascular density and reducing microvascular perfusion [12-15]. Furthermore, microcirculatory oxygen delivery relies on both microvascular density and microvascular perfusion [12]. That changes in microcirculatory perfusion could be important during hypovolemia has been shown by Dubin et al. who have identified that depressed sublingual and intestinal microcirculatory perfusion in hemorrhaged sheep was associated with intramucosal acidosis [16]. Furthermore, regional perfusion is regulated by, e.g., arterial and pulmonary baroreceptors, carotid sinus, and muscle sympathetic nerve activity in order to maintain perfusion of vital organs [17]. In this respect, application of LBNP leads to a shift from blood volume away from the upper body and thereby triggers the autonomic baroreflex activation [18].

Our group has recently employed near-infrared spectroscopy (NIRS) during LBNP to study peripheral tissue oxygenation under conditions of controlled central hypovolemia in a group of healthy volunteers [19]. In agreement with Soller et al. [8], we found that NIRS could be used to detect changes in peripheral tissue hemoglobin content (THI) and tissue oxygenation (StO2) consequent to central hypovolemia. To integratively assess the response of the microcirculation to controlled central hypovolemia, sublingual SDF imaging and NIRS in combination with a VOT were performed at 0 and -60 mmHg LBNP in a subset of this group. SDF images were analyzed for total vessel density (TVD), perfused vessel density (PVD), and the microvascular flow

index (MFI) and flow heterogeneity (MFIhetero), thus, incorporating both diffusion- and convection-related microcirculatory parameters. VOT-derived NIRS traces were analyzed for parameters reflecting tissue oxygen consumption and post-occlusion microvascular reperfusion and hyperemia. Using this methodology we were able to test the hypothesis that controlled, adequately compensated, central hypovolemia (i.e. hypovolemia without severe hypotension) in subjects with intact autoregulation would be associated with decreased microcirculatory diffusion (TVD and PVD) and convection (MFI and MFIhetero) properties and, consequently, decreased tissue oxygen carrying capacity (THI) and tissue oxygenation (StO2). Furthermore, we aimed to evaluate the impact of hypovolemia-induced microcirculatory alterations on resting tissue oxygen consumption.

MATERIALS & METHODS

Subjects

The sublingual SDF image acquisition and NIRS measurements in combination with a VOT performed at 0 and -60 mmHg LBNP were part of an experimental protocol described previously [19]. The study protocol was approved by the medical ethics committee of the Academic Medical Center of the University of Amsterdam. Written informed consent was obtained from all subjects and subjects refrained from the intake of vaso-active medication and intensive exercise prior to the experiment. In order to minimize possibly confounding factors such as gender, age and physical fitness, which contribute to different LBNP tolerances [11], we recruited a homogenous group of healthy male volunteers. Twenty-four healthy male subjects with a mean±standard error of the mean (SEM) age of 28±1 years, body weight of 82±2 kg, and height of 182±1 cm volunteered for the study and passed an exercise electrocardiogram test and medical history questionnaire [19].

Lower Body Negative Pressure

The LBNP device and protocol have been described in detail elsewhere [19]. In brief, the lower body of participants was positioned in a negative pressure chamber and an airtight seal at the level of the iliac crest was applied. The LBNP protocol consisted of a 30 min baseline period at LBNP=0 mmHg followed by stepwise application of LBNP to -20, -40, and -60 mmHg. Thenar and forearm StO2 and THI values were continuously recorded and at baseline and at -60 mmHg LBNP, sublingual SDF images were acquired and a VOT was performed. The protocol could be immediately terminated by the physician in case of impending cardiovascular collapse or at the request of the subject. Impending cardiovascular collapse was defined by a drop in systolic blood pressure of > 15 mmHg from baseline blood pressure, bradycardia or if termination was requested by the subject due to dizziness, discomfort or nausea. An attending physician was present in the laboratory during all experiments to ensure safety of participants.

Hemodynamic monitoring

To monitor hemodynamic changes induced by LBNP, the SV, HR, systemic vascular resistance (SVR), cardiac output (CO), and mean arterial pressure (MAP) were continuously and non-invasively measured throughout the entire protocol using volume clamp finger plethysmography (Nexfin, BMEYE, Amsterdam, the Netherlands). The finger cuff of the monitor was placed around the right middle finger and was kept at heart level. Nexfin and its predecessors have been intensively validated and have shown to provide reliable measurements of hemodynamic parameters [20-22]

Sidestream dark field imaging

Sublingual microcirculatory density and perfusion were monitored using an SDF imaging device (Microvision Medical, Amsterdam, the Netherlands) at 0 (baseline) and -60 mmHg LBNP. A detailed description of the SDF technology is provided elsewhere [23]. Briefly, in SDF imaging, the tissue is illuminated with green light emitting diodes concentrically placed around the central microscope objective to provide SDF illumination. The lens system in the core of the objective is optically isolated from the illuminating outer ring to prevent image contamination by tissue surface reflections, which allows imaging of subsurface microcirculatory structures. The lens of the SDF device is covered by a sterile disposable cap (Microscan Lens, MicroVision Medical, Amsterdam, The Netherlands). The video microscope uses an x5 objective and monitors a mucosal area of 1.0 x 0.75 mm at a 720 x 567 pixel resolution and a frame rate of 25 Hz.

SDF images were acquired by gently placing cap-covered imaging probe on the sublingual mucosal tissue. Care was taken to prevent pressure artifacts [24,25]. According to the round-the-table-conference guidelines, five different mucosal sites were measured per time point [26]. The obtained video sequences were stored on DVI tape and saved on a computer in DV-AVI file format and analyzed by two analysts (SAB and MR; blinded to the subject and time point) for total vessel density (TVD; mm vessel/mm^2 image), perfused vessel density (PVD; mm perfused vessel/mm^2 image), and the microvascular flow index (MFI; 0=no flow, 1=intermittent flow, 2=sluggish flow, 3=normal flow) using a dedicated computer software package (Automated Vascular Analysis, Microvision Medical) [27,28]. The MFI score was calculated by averaging the MFI scores over four quadrants per image and 5 images per time point (=20 scores per time point per subject). The MFIhetero score was calculated as the highest MFI score minus the lowest MFI score, divided by the mean MFI score across all 20 MFI scores per time point per subject [24]. Microcirculatory analysis was confined to vessels with an average diameter below 25 µm.

Near-infrared spectroscopy

Two InSpectra tissue spectrometers (Hutchinson Technology, Hutchinson, MN) were used to measure StO2 and THI on the left forearm and thenar continuously and non-invasively as described previously [19]. Both NIRS devices were equipped with a reflectance mode probe having a 1.5 mm optical fiber to illuminate tissue and

a 0.4 mm optical fiber to collect the backscattered light. The spacing between the illuminating fiber and the detection fiber was 15 mm and the NIRS measurement depth is estimated as half this probe spacing [29,30]. During the entire protocol, hand and forearm were kept at heart level and subjects were instructed to prevent movement of hand or arm.

Vascular occlusion test

During a VOT, which was performed at 0 (baseline) and -60 mmHg LBNP, the forearm and hand circulation is transiently stopped by arterio-venous occlusion induced by application of suprasystolic pneumatic pressure around the upperarm [31,32]. As demonstrated by Myers and colleagues, baseline StO2 represents the balance between oxygen delivery rate and the oxygen consumption rate, whereas THI measures the hemoglobin content in the NIRS measurement volume and represents the tissue oxygen carrying capacity [29,30]. Although related to the systemic hemoglobin content, THI is also influenced by microvascular density and tone [29,32]. In the absence of oxygen delivery (i.e., during vascular occlusion), the StO2 downslope provides an indication of the microcirculatory deoxygenation rate. Multiplication of the mean tissue oxygen carrying capacity (THI) and microcirculatory deoxygenation rate (StO2 downslope) provides an index for the resting tissue oxygen consumption rate per minute (iVO2= (THI bsln*StO2 blsn)-(THI min*StO2 min)/3), a method which corresponds to the method used by Skarda et al. [32]. After release of the occlusion, the StO2 upslope is considered to represent the microvascular reperfusion rate and the subsequent suprabaseline StO2 levels is considered to represent reactive hyperemia [33,34]. Hence, using a VOT in combination with NIRS allows the characterization of tissue oxygen consumption and microvascular reperfusion and reactivity.

After a 2-min stabilization period (baseline) at 0 and -60 mmHg LBNP, stagnant ischemia was induced for 3 min by rapidly inflating a pneumatic cuff (within 5 sec), placed around the upperarm, to 50 mmHg above systolic blood pressure. Subsequently, the cuff was deflated (within 1 sec) and StO2 measurements continued up to 5 min post-ischemia. Data were continuously saved on two computers and analyzed using InSpectra Analysis V3.3 software (Hutchinson Technology). The VOT-derived StO2 traces were divided into four phases: baseline, ischemia, reperfusion, and hyperemia. The ischemic phase was analyzed for StO2 and THI downslope. The reperfusion phase was analyzed for StO2 and THI upslope and the hyperemic phase was analyzed for the StO2 and THI area under the hyperemic curve (AUC) [31].

Data analysis

Data were plotted and analyzed using GraphPad Prism software (GraphPad Prism Software, San Diego, CA). Data are reported in median (25^{th}-75^{th} pct) in the tables and as percentage of baseline in the text. Comparative analysis of data sets between baseline and LBNP=-60 mmHg was performed using Wilcoxon matched pairs test. $P<0.05$ was considered statistically significant.

RESULTS

Of the 24 subjects, 23 reached the LBNP=-60 mmHg level [19], but only 14 tolerated the LBNP=-60 mmHg step long enough to complete the VOT and SDF image acquisition. The hemodynamic parameters measured before and during application of LBNP in these subjects are presented in Table 1.

Table 1. Hemodynamic parameters before and during application of lower body negative pressure (LBNP). Values are reported in median (25^{th}-75^{th}).

		LBNP=0 mmHg	LBNP=-60 mmHg	Delta (% of baseline)	p-value
Hemodynamic parameters					
Stroke volume	mL	117 (108-126)	77 (71-87)	-34	0.001
Heart rate	bpm	59 (52-66)	83 (76-88)	41	0.001
System vascular resistance	AU	1063 (951-1136)	1153 (1020-1307)	8	0.035
Cardiac output	L/min	6.8 (6.2-7.6)	6.0 (5.8-7.1)	-13	0.033
Mean arterial pressure	mmHg	92 (84-96)	93 (86-97)	1	0.230

LBNP of -60 mmHg induced a significant 34 % decrease in SV (p=0.001), which in turn, led to a significant 41 % increase in HR (p=0.001) and an 8 % increase in SVR (p=0.035). CO decreased by 13 % (p=0.033), but MAP was maintained around baseline level (p=0.230). Hence, LBNP caused a significant shift in blood volume from the upper to the lower body, which was accompanied by a decrease in SV and activation of compensatory mechanisms such as increased HR and SVR to maintain the MAP.

The microcirculatory parameters derived from the SDF images obtained at 0 (baseline) and -60 mmHg LBNP are presented in Table 2. Typical SDF images obtained at 0 and -60 mmHg LBNP are shown in Figure 1. LBNP induced a slight decrease in

Table 2. Sidestream dark field (SDF) imaging parameters before and during application of lower body negative pressure (LBNP). Values are reported in median (25^{th}-75^{th}).

		LBNP=0 mmHg	LBNP=-60 mmHg	Delta (% of baseline)	p-value
Sidestream dark field (SDF) imaging parameters					
Total vessel density	mm/mm^2	13.3 (12.7-14.2)	12.2 (11.6-13.7)	-8	0.070
Perfused vessel density	mm/mm^2	13.2(12.5-14.1)	12.2 (11.5-13.6)	-8	0.035
Portion of perfused vessels	%	99 (98-100)	99 (97-99)	0	0.241
Microvascular flow index	AU	3.0 (2.8-3.0)	2.8 (2.6-2.9)	-7	0.014
Flow heterogeneity index	AU	0.3 (0-0.4)	0.4 (0.4-0.8)	33	0.003

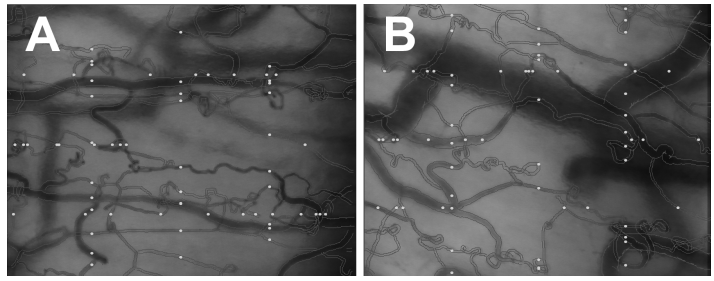

Figure 1. Typical sidestream dark field images of the sublingual microcirculation acquired at 0 **(A)** and -60 **(B)** mmHg lower body negative pressure.

TVD (8 %, p=0.070) and PVD (8 %, p=0.035). The portion of perfused vessels (=PVD/TVD) remained unaltered (p=0.241).

The MFI was also slightly decreased (7 %, p=0.014) as a result of LBNP and the MFIhetero was significantly increased (33 %, p=0.003). This indicates that both diffusion- and convection-related properties of the microcirculation are affected during controlled, adequately compensated, central hypovolemia.

The StO2 and THI parameters derived from the VOT performed before and during application of LBNP are presented in Table 3. Typical forearm StO2 traces during a VOT performed at 0 and -60 mmHg LBNP are shown in Figure 2.

Table 3. Baseline and vascular occlusion test-derived tissue oxygen saturation (StO2) and tissue hemoglobin index (THI) parameters before and during application of lower body negative pressure (LBNP). Values are reported in median (25^{th}-75^{th}).

		LBNP=0 mmHg	LBNP=-60 mmHg	Delta (% of baseline)	p-value
Thenar StO2 and THI during the vascular occlusion test					
StO2 baseline	%	90 (84-91)	86 (84-89)	-4	0.023
THI baseline	AU	13.5 (12.7-14.6)	12.7 (12.0-14.1)	-6	0.009
StO2 downslope	%/min	-10.5 (8.8-11.8)	-10.3 (8.8-13.4)	2	0.542
THI downslope	AU/min	-0.95 (0.75-1.20)	-1.03 (0.87-1.23)	8	0.542
iVO2	AU*%/min	135 (119-164)	134 (105-164)	-1	1.000
StO2 upslope	%/min	153 (91-183)	159 (138-174)	4	0.268
THI upslope	AU/min	18 (15-22)	22 (17-26)	22	0.091
StO2 area under the curve	%*min	11.6 (8.5-15.3)	14.3 (11.3-16.0)	23	0.426
THI area under the curve	AU*min	3.9 (1.9-5.0)	4.4 (3.3-6.1)	13	0.104
Forearm StO2 and THI during the vascular occlusion test					
StO2 baseline	%	81 (73-85)	78 (70-81)	-4	<0.001
THI baseline	AU	6.8 (5.1-7.5)	5.8 (4.7-6.5)	-15	<0.001
StO2 downslope	%/min	-7.9 (6.3-10.0)	-10.1 (7.4-13.1)	28	0.042

Table 3. (continued)

		LBNP=0 mmHg	LBNP=-60 mmHg	Delta (% of baseline)	p-value
THI downslope	AU/min	-0.17 (0.10-0.53)	-0.22 (0.12-0.41)	-29	0.890
iVO2	AU*%/min	47 (31-62)	50 (39-69)	6	0.952
StO2 upslope	%/min	137 (104-198)	155 (131-230)	13	0.049
THI upslope	AU/min	8.4 (4.6-11.3)	12.0 (7.1-22.9)	43	0.577
StO2 area under the curve	%*min	9.1 (6.1-11.0)	13.4 (9.9-17.4)	47	0.025
THI area under the curve	AU*min	0.8 (0.6-1.6)	1.4 (1.0-2.4)	75	<0.001

In the thenar, StO2 decreased by 4 % (p=0.023) and THI decreased by 6 % (p=0.009). In the forearm, StO2 decreased by 4 % (p<0.001) and THI decreased by 15 % (p<0.001). In the thenar, VOT-derived StO2 and THI parameters did not change significantly NP (p=0.091). In the forearm, in contrast, all VOT-derived StO2 parameters changed significantly: StO2 downslope increased by 28 % (p=0.042), StO2 upslope increased by 13 % (p=0.049), and StO2 area under the hyperemic curve increased by 47 % (p=0.025). The forearm THI downslope (p=0.890) and upslope (p=0.557) did not change significantly, but the THI AUC increased by 75 % (p<0.001). Although the StO2 downslope increased by 28 %, the resting tissue oxygen consumption rate (iVO2) remained unaffected by application of LBNP (p=0.952), indicating the importance of combining StO2 and THI data for proper interpretation of the NIRS results with respect to the assessment of tissue oxygen consumption rate. Taking the VOT results into account, the forearm NIRS measurements were more sensitive to hypovolemia than the thenar NIRS measurements.

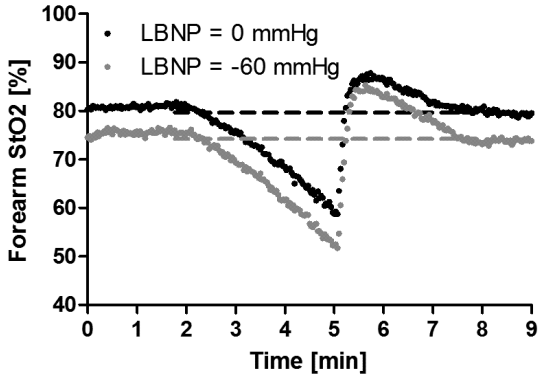

Figure 2. Typical forearm tissue oxygen saturation (StO2) traces during a vascular occlusion test performed at 0 and -60 mmHg lower body negative pressure (LBNP).

DISCUSSION

The aim of this study was to test the hypothesis that controlled, adequately compensated, central hypovolemia in subjects with intact autoregulation would be associated with decreased microcirculatory diffusion (TVD and PVD) and convection (MFI and MFIhetero) properties and, consequently, decreased tissue oxygen carrying capacity (THI) and tissue oxygenation (StO2). Furthermore, we aimed to evaluate the impact of hypovolemia-induced microcirculatory alterations on resting tissue oxygen consumption. To this end, we performed sublingual SDF imaging and NIRS in combination with a VOT in an LBNP model. To compensate for the central hypovolemia induced by LBNP, both HR and SVR increased, in agreement with studies by Convertino et al. [5-9]. In general, the increased HR and SVR compensated adequately for the decreased SV and maintained the MAP around baseline level during the entire LBNP protocol. It must be noted, however, that cardiovascular collapse (i.e., rapid decline of MAP) was not included in the analysis as this fell out of the scope of the study. The primary findings of the present study were that controlled, adequately compensated, central hypovolemia led to: 1) significantly decreased sublingual microvascular density (PVD) and perfusion (MFI and MFIhetero) and consequently, 2) significantly decreased tissue oxygen carrying capacity (THI) and tissue oxygenation (StO2); and 3) an unaltered resting tissue oxygen consumption rate (iVO2).

One of the findings in the present study was that both diffusion- and convection-related properties of the microcirculation are affected during hypovolemia; i.e., both microvascular density (PVD) and perfusion (MFI and MFIhetero) were significantly affected by application of LBNP. The decreased microvascular density during application of LBNP confirms the findings in an earlier study by Ward et al. where SDF images obtained during LBNP were analyzed for PVD [7]. In that study, however, the authors did not analyze alterations in microcirculatory flow characteristics, which is potentially a significant variable since microcirculatory oxygen delivery relies on both microvascular density and microvascular perfusion and downregulation of the microcirculation for maintenance of blood pressure might affect one or both of these microcirculatory properties [12-15]. Activation of the autonomic baroreflex by the reduction in upper body blood volume consequent to the application of LBNP leads to a downregulation of the peripheral microcirculation by increasing peripheral vascular resistance. Although we have not measured muscle sympathetic nerve activity and/or baroreceptor receptors in the present study, previous research by others has demonstrated that application of LBNP results in several effects on cardiac and arterial receptors [35] and stimulus of the arterial and pulmonary receptors leads to peripheral vasoconstriction [36]. These findings support the observed decrease in both microcirculatory diffusion and convection in response to the reduced macrocirculatory volume in the upper body.

Sublingual SDF imaging has been used in many clinical scenarios, including cardiogenic shock, sepsis, and surgery [37-40]. Dubin et al., furthermore, have shown that depressed microcirculatory perfusion in hemorrhaged sheep was associated with intramucosal acidosis, emphasizing the relevance of alterations in microcirculatory

perfusion in hypovolemia [16]. These studies therefore underscore the relevance of sublingually-obtained parameters of microvascular density and perfusion.

In addition to the decreased microvascular density (PVD) and mean microcirculatory flow (MFI), we found that microcirculatory flow was heterogeneously affected by LBNP as indicated by the increased MFIhetero. In a recent study by Trzeciak et al., early sublingual microcirculatory perfusion derangements were studied in patients with severe sepsis/septic shock [24]. In the present study, the MFIhetero score at LBNP=0 mmHg is similar to the score reported by Trzeciack et al. for the healthy control subjects and the MFIhetero score at LBNP=-60 mmHg is similar to the score for the surviving patients. Trzeciak et al. further reported that non-surviving patients had an MFIhetero score twice as high as the surviving patients [24], underlining the pathophysiological relevance of this parameter.

It is important to note that the NIRS measurement comprises arteriolar, capillary, and venular blood. Since LBNP leads to a reduction in microvascular density and perfusion, which is regulated at the arterial/arteriolar side of the microcirculation, the contribution of this side to the NIRS signal most likely decreases during LBNP. This would lead to a reduction in StO2 as observed in the present study. The reduction in microcirculatory density and perfusion was reflected by the decreased steady-state (i.e., baseline) StO2 and THI values during application of LBNP as presented previously by us [19] and others [7-9]. A potential physiological explanation for the difference between the thenar and forearm in terms of sensitivity to hypovolemia is that the circulation in the hands might be well-preserved during cardiovascular challenges such as the activation of the baroreflex during application of LBNP. This is also observed, for instance, during severe hypothermia where a phenomenon termed cold-induced vasodilation opens the (micro)circulation to the hands in order to prevent cold-induced necrosis and thereby increase the chances of survival [41]. However, whether such a response would actually overrule the baroreflex remains unknown.

Although Soller et al. also found a reduction in forearm StO2 with increasing LBNP, care should be taken when comparing StO2 values. The values we present here are significantly higher than those presented by Soller et al, probably because different NIRS devices were used, as explained in more detail in [8]. Furthermore, in the studies where Soller et al employed the same NIRS device as we have done here, the probes were placed in the thenar and not on the forearm. Similarly to the findings in the present study, the authors reported no significant differences in StO2 and THI baseline values in the thenar. In the forearm, in contrast, the authors report on a significant reduction in NIRS parameters, although measured using different NIRS technology. Hence, the observed reduction forearm StO2 and THI in response to application of LBNP in both studies is comparable, demonstrating the sensitivity of this application site in healthy volunteers.

In the present study we applied a VOT to measure potential alterations in tissue oxygen consumption and showed that this was maintained even though steady-state microcirculatory oxygenation was reduced. Our finding of maintained oxygen consumption measured locally in the forearm and thenar tissue is in agreement of the observations by Ward et al. on maintained systemic oxygen consumption during LBNP

[7]. Considering the extent of the observed hypovolemia-induced microcirculatory alterations and the low basal oxygen consumption rate of the resting skeletal muscle, this is not a very surprising result [42].

In this light, it is important to realize that LBNP is a model of controlled central hypovolemia rather than a model simulating hemorrhage-induced hypovolemia. In hemorrhage, both coagulation and inflammation pathways are activated which significantly affect microvascular function [4]. Using an LBNP model, we were successful in studying the microcirculatory response to controlled, adequately compensated, central hypovolemia in case of intact autoregulation. Hence, we have delineated the microcirculatory alterations in response to (central) hypovolemia from the microcirculatory alterations in response to activation of coagulation and inflammation pathways. The results we present, however, are therefore not directly applicable in hypovolemic patients where microvascular function might be compromised due to the underlying pathophysiological conditions. In line, the present study therefore underscores the major roles of inflammation and coagulation disorders and shows that hypovolemia is only minimally responsible for the depressed microcirculation observed in critically ill patients.

The THI represents the amount of hemoglobin present in the NIRS measurement volume, which depends on both the systemic hemoglobin level and the peripheral (micro)vascular tone. As hemoglobin is the only source of oxygen for the tissue cells (myoglobin deoxygenation occurs at much lower saturations), the THI reflects the tissue oxygen carrying capacity. The StO2 downslope, on the other hand, represents the rate at which the hemoglobin present in the NIRS measurement volume deoxygenates during ischemia. Hence, if less hemoglobin would be present in the tissue, deoxygenation of the hemoglobin would be more rapidly, leading to a steeper StO2 downslope. The StO2 downslope in the ischemic phase of the VOT (i.e., during vascular occlusion), which reflects the microcirculatory deoxygenation rate, is steeper during application of LBNP. This did not mean that the tissue oxygen consumption rate was higher. Indeed, if one would only measure the StO2 downslope and interpret this as a measure of tissue oxygen consumption rate, erroneous conclusions could be drawn since the tissue oxygen carrying capacity (THI) could change between two measurement time points. This is exemplified in the present study where the StO2 downslope increased by 28 % while the resting tissue oxygen consumption remained unaffected by application of LBNP. Since oxygen consumption is not limited by the amount of available oxygen and the diffusion of oxygen is a passive process, maintained oxygen consumption under conditions of reduced oxygen delivery directly reduces the StO2. Here we thus demonstrate that multiplication of the tissue oxygen carrying capacity (THI) by the microcirculatory deoxygenation rate (StO2 downslope) is required to find an index for the resting tissue oxygen consumption rate (iVO2) and that combining StO2 and THI data is key for proper interpretation of NIRS results under compromised circulatory conditions. However, it is important to point out that this method of calculating oxygen consumption has not been validated in humans and should be interpreted with care.

Additional findings

During vascular occlusion, a slight decrease in THI (THI downslope) was observed that could reflect ischemia-induced vasoconstriction that caused redistribution of blood from the microcirculation to the larger vasculature, thereby eliminating this blood from the NIRS measurement vasculature. The THI downslope did not change during our LBNP protocol, but could perhaps be affected under pathophysiological conditions where (micro)vascular regulation (and thus ischemia-induced vasoconstriction) is disturbed.

The post-occlusion StO2 and THI upslope and AUC are considered to be parameters reflecting microvascular reperfusion and reactive hyperemia, respectively [31-34]. We have shown that the upslope and AUC were unaltered in the thenar and increased in the forearm. This discrepancy could be caused by different volume re-distribution to the different compartments, which requires further research. The increase in microvascular reperfusion and reactivity in the present study is probably explained by the decreased baseline StO2 and increased StO2 downslope as a result of LBNP-induced hypovolemia, which led to an increased ischemic hit during vascular occlusion [43]. This, in turn, triggered excessive reactive hyperemia in our healthy volunteers with intact vasoregulatory mechanisms [33,34]. However, it must be noted that the (patho)physiological mechanisms underlying (alterations in) StO2 and THI upslope and AUC warrant further study.

Study limitations

We acknowledge that the present study design has an important limitation being that it consists of only two time points, whereas more time points could show a trend in hypovolemia-related alterations in SDF imaging and NIRS-derived parameters. However, the time required for SDF image acquisition at five sublingual sites plus the VOT does not allow multiple time points due to the duration-dependent tolerance for LBNP [11]. Furthermore, other techniques for monitoring microvascular flow, such as laser Doppler velocimetry, were not included in the present study. As the alterations in microcirculatory density and perfusion as measured using SDF imaging were of modest extent, inclusion of other techniques could potentially provide additional information on the microcirculatory response to application of LBNP. Nonetheless, with the present study design we were able to detect significant alterations in SDF imaging parameters of microvascular density and perfusion and NIRS-derived parameters of tissue oxygenation, microvascular reperfusion, and reactive hyperemia.

CONCLUSIONS

By integrative assessment of microcirculatory alterations in response to controlled central hypovolemia using SDF, NIRS, and the VOT, we confirmed our study hypothesis that controlled, adequately compensated, central hypovolemia in subjects with intact autoregulation would be associated with decreased microcirculatory diffusion (PVD) and convection (MFI and MFIhetero) properties and, consequently, decreased tissue oxygen carrying capacity (THI) and tissue oxygenation (StO2). Using a VOT, moreover,

we showed that resting tissue oxygen consumption was maintained under conditions of adequately compensated central hypovolemia. We found that combining StO2 and THI data is key for the proper interpretation of the NIRS results with respect to the assessment of tissue oxygen consumption rate and that forearm NIRS measurements are more sensitive to hypovolemia compared to thenar NIRS measurements, both with and without a VOT. This is, to our knowledge, the first study linking parameters of microvascular density (TVD and PVD) and perfusion (MFI and MFIhetero) obtained using SDF imaging to parameters of tissue oxygen carrying capacity (THI) and tissue oxygenation (StO2) obtained using NIRS during controlled central hypovolemia. Moreover, this is the first study analyzing both StO2 and THI dynamics during a VOT.

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IV

MULTI-SITE AND MULTI-DEPTH NEAR-INFRARED SPECTROSCOPY IN A MODEL OF SIMULATED (CENTRAL) HYPOVOLEMIA: LOWER BODY NEGATIVE PRESSURE

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ABSTRACT

To test the hypothesis that the sensitivity of near-infrared spectroscopy (NIRS) to reflect the degree of (compensated) hypovolemia would be affected by the application site and probing depth. We simultaneously applied multi-site (thenar and forearm) and multi-depth (15-2.5 mm and 25-2.5 mm probe distance) NIRS in a model of simulated hypovolemia: lower body negative pressure (LBNP).

Twenty-four healthy male volunteers were subjected to an LBNP protocol in which a baseline (bsln) period of 30 minutes was followed by a stepwise manipulation of negative pressure from 0 to -20, -40, -60, -80, and -100 mmHg (5 min per step). Stroke volume and heart rate were measured using volume-clamp finger plethysmography. Two multi-depth NIRS devices were used to measure tissue oxygen saturation (StO2) and tissue hemoglobin index (THI) continuously in the thenar and the forearm. To monitor the shift of blood volume towards the lower extremities, calf THI was measured by single-depth NIRS.

The main findings were that the application of LBNP resulted in a significant reduction in stroke volume which was accompanied by a reduction in forearm StO2 and THI.

In conclusion, NIRS can be used to detect changes in StO2 and THI consequent to central hypovolemia. Forearm NIRS measurements are more sensitive to hypovolemia than thenar NIRS measurements. The sensitivity of these NIRS measurements does not depend on NIRS probing depth. The LBNP-induced shift of blood volume is reflected by the decreased THI in the forearm and an increased THI in the calf.

INTRODUCTION

Hypovolemia is a major complication occurring in numerous clinical scenarios involving civilian, combat, and disaster field traumas, surgical injuries, and intensive care settings [1-2]. The reduction in stroke volume (SV) associated with hypovolemia is physiologically countered by compensatory mechanisms such as increasing heart rate (HR) and systemic vascular resistance (SVR) to prevent the consequent decrease in blood pressure (BP) and organ perfusion. When these mechanisms fail, however, BP will fall and adequate perfusion of vital organs will be at risk. Hence, detection of hypovolemia before BP falls is of prime importance to initiate and/or guide treatment strategies aimed at maintaining adequate cardiac output (CO) and BP in the prevention of organ hypoperfusion [3-4].

To this end, near-infrared spectroscopy (NIRS) has been widely explored, successfully and unsuccessfully, for measuring tissue oxygen saturation (StO2) and an index for tissue hemoglobin content (THI) in attempts to identify the presence and severity of hypovolemia [5-10]. However, the main problem with the interpretation of NIRS data from these studies is the diversity of methodologies used for the assessment of StO2 and THI. Two critical aspects concerning the methodology have been identified: the application site and the probing depth [7,10]. The application site is important as differences may exist in the sensitivity of underlying muscle groups and other tissues to cardiovascular challenges such as central hypovolemia. In addition, the probing depth, which correlates directly with the NIRS probe size, determines the relative contribution of muscular and (sub)dermal tissue to the NIRS measurement [7,10].

In an attempt to evaluate the detection of hypovolemia using NIRS, NIRS has recently been employed in a lower body negative pressure (LBNP) model of simulated central hypovolemia. Thenar StO2 was measured using a second-derivative NIRS device equipped with a 15 mm probe and forearm StO2 was measured using a multi-depth NIRS device equipped with a 30 mm probe [8]. No change in StO2 in the thenar was found, while forearm StO2 significantly decreased. It was suggested that the forearm was a superior application site for the detection of hypovolemia compared to thenar and that the 15 mm probe size was too small to collect light from a sufficiently deep layer of muscular tissue and thus not able to detect hypovolemia. We criticized the applied methodology because the thenar and forearm measurements were performed in separate experiments rather than simultaneously and because the study did not investigate the effects of the 15 mm probe on the forearm or of the 30 mm probe on the thenar [12]. Consequently, whether the superior detection of hypovolemia in the forearm was due to a more sensitive application site or due to greater probing depth remained inconclusive.

The aim of the present study was to test the hypothesis that the sensitivity of near-infrared spectroscopy (NIRS) to reflect the degree of (compensated) hypovolemia would be affected by the application site and probing depth. For this purpose, we simultaneously applied multi-site (thenar and forearm) and multi-depth (15 mm and 25 mm probe) NIRS during LBNP as a model of central hypovolemia.

MATERIALS AND METHODS

Subjects

Twenty-four healthy male volunteers participated in this study. The study guidelines and procedures were reviewed and approved by the Medical Ethics Committee of the Academic Medical Center of the University of the Amsterdam and voluntary written informed consent was obtained from all subjects. All participants were screened by a cardiologist, underwent an exercise electrocardiogram (ECG test), and completed a medical history questionnaire prior to inclusion. All subjects were instructed to refrain from caffeine intake and other autonomic stimulants and to abstain from excessive physical labor >8 hours prior to the LBNP experiment.

LBNP protocol and measurements

In brief, the LBNP protocol consisted of a baseline period of 30 min followed by stepwise manipulation of negative pressure from 0 to -20, -40, -60, -80, and -100 mmHg (5 min per step). The LBNP protocol could immediately be terminated by the subject or attending physician in case of impending cardiovascular collapse or per request of the subject. Cardiovascular and NIRS parameters were recorded at the end of each LBNP step and just before the onset of cardiovascular collapse. To monitor the shift of blood volume away from the upper body, thoracic bioimpedance (NICOM, Cheetah Medical Inc., Wilmington, DE) was used to measure thoracic fluid content (TFC) which was expected to decrease when blood is shifted away from the upper body [13-15]. Additionally, to monitor the shift of blood volume towards the lower body, a single-depth 15 mm NIRS probe (InSpectra, Hutchinson Technology, Hutchinson, MN) was placed on the medial soleus of the left calf to monitor the calf THI which was expected to increase when blood is shifted towards the lower body [9]. To monitor the physiological changes associated with LBNP, CO, SV, HR, mean arterial pressure (MAP), and SVR were continuously and non-invasively measured using volume-clamp finger plethysmography (Nexfin monitor, BMEYE, Amsterdam, the Netherlands) [16-18]. Multi-depth NIRS was employed to measure StO2 and THI in the thenar and forearm during LBNP. Both the StO2 and the THI calculation from the NIRS signal have been validated by Myers et al. [19-23]. Two multi-depth NIRS devices (InSpectra, Hutchinson Technology, Hutchinson, MN) were used to measure StO2 and THI continuously and non-invasively in the left thenar eminence and in the lateral side of the anterior surface of the left forearm, as described previously [11,21]. We refer to the NIRS measurements performed on the forearm and thenar with the 15 mm and 25 mm probe spacing as F_{15}, F_{25}, T_{15}, and T_{25}, respectively.

Statistical analysis

Data plotting and analysis were performed in GraphPad Prism software (GraphPad Software, San Diego, CA, USA). Data are presented as mean±standard error of the mean (SEM) after the normal distribution of the data was confirmed by D'Agostino and Pearson normality tests. First TFC, calf THI, and SV were plotted versus the level of LBNP and statistical significance of differences between LBNP levels was analyzed

using a one-way analysis of variance (ANOVA) for repeated measurements with a Bonferroni post-hoc test. P<0.05 was considered statistically significant. Second, all data were normalized and expressed as percentage of baseline value (bsln). As the decrease in SV consequent to LBNP is the primary stimulus for physiological compensatory mechanisms to be activated (e.g., increased HR and SVR), the data were plotted versus SV [% of bsln] and, to allow statistical analysis using ANOVA, data were categorized according to the following pre-defined categories: SV=100 % of bsln, SV=100-80 % of bsln, SV=80-60 % of bsln, and SV=60-40 % of bsln. Furthermore, Pearson's correlation analysis was performed for forearm StO2 [% of blsn] and THI [% of blsn] versus SV [% of blsn].

RESULTS

Subject characteristics

Since the tolerance of subjects to LBNP and the extent of the cardiovascular responses depend, amongst other factors, on age, physical fitness, gender, body size, and hydration status [24-29], a homogenous subject population was recruited for this study. The study population consisted of 24 male subjects with a mean±SEM age of 28±1 years, body weight of 82±2 kg, and height of 182±1 cm.

LBNP-induced blood volume shift and stroke volume reduction

All 24 subjects completed the LBNP=-20 and -40 mmHg steps, 23 subjects completed the LBNP=-60 mmHg step, 15 subjects completed the LBNP=-80 mmHg step, and 9 subjects completed the LBNP=-100 mmHg step. Figure 2 shows that in all subjects, TFC decreased significantly (p<0.05) and calf THI increased significantly (p<0.05) with increasing LBNP. The decrease in TFC was associated a significant decrease in

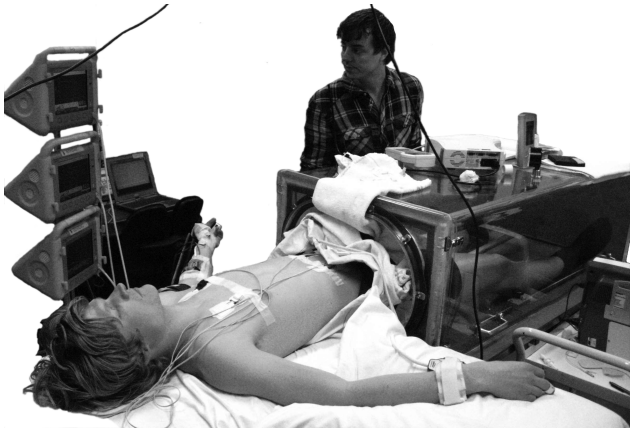

Figure 1. A subject placed in the lower body negative pressure chamber.

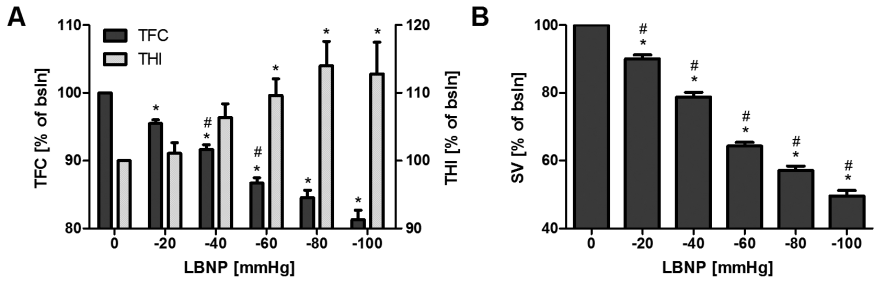

Figure 2. A) Thoracic fluid content (TFC) and calf tissue hemoglobin index (THI), and **B)** stroke volume (SV) during stepwise increase of lower body negative pressure (LBNP), normalized and expressed as percentage of baseline (bsln). * p<0.05 versus LBNP = 0 mmHg and # p<0.05 versus previous LBNP step.

SV (P<0.05). As the decrease in SV consequent to LBNP was the primary trigger for compensatory mechanisms activation, data were categorized according to SV=100 % of bsln (n=24), SV=100-80 % of bsln (n=36), SV=80-60 % of bsln (n=34), and SV=60-40 % (n=25).

Hemodynamic response

In response to the decrease in SV, HR and SVR increased. At mild central hypovolemia induced by LBNP, HR remained unchanged (p>0.05), but when hypovolemia progressed, HR increased significantly (p<0.05). SVR increased significantly (p<0.05) from baseline to mild central hypovolemia, but remained unchanged (p>0.05) during further reduction of SV. During the entire LBNP protocol, with exclusion of cardiovascular collapse, CO and MAP maintained around baseline level (p>0.05).

Multi-site and multi-depth NIRS

All NIRS results, expressed as percentage of baseline value, are shown in Figure 3. In general, forearm NIRS measurements are more sensitive to reflect changes in SV than thenar NIRS measurements and the sensitivity of these measurements does not depend on the NIRS probing depth. Where HR did not show a significant change in the first SV category of SV=100-80 % of bsln, forearm and thenar StO2 and THI did for both probing depths. However, changes measured on the thenar were of marginal extent.

DISCUSSION

In the present study we have simultaneously applied multi-site and multi-depth NIRS to test the hypothesis that the sensitivity of NIRS to reflect the degree of (compensated) hypovolemia would be affected by the application site and probing depth. The main findings were that 1) the application of LBNP resulted in a significant shift in blood volume from the upper body to the lower body accompanied by a reduction in SV,

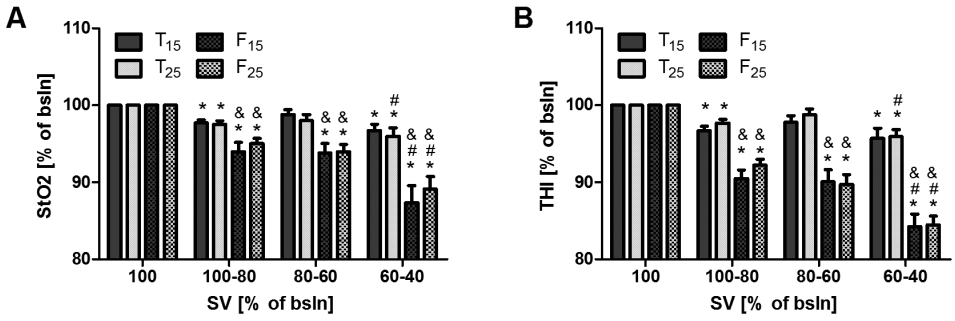

Figure 3 A) Thenar and forearm tissue oxygen saturation (StO2) and **B)** thenar and forearm tissue hemoglobin index (THI) plotted versus stroke volume (SV). All parameters were normalized and expressed as percentage of baseline (bsln). * p<0.05 versus SV=100 % of bsln, # p<0.05 versus one SV category higher, and & p<0.05 versus same probe on the thenar. P>0.05 for 15 mm versus 25 mm.

2) this SV reduction consequent to LBNP was countered by an increase in HR and SVR, which adequately compensated for the decrease in SV and maintaining CO and MAP near baseline level, 3) NIRS can be used to detect changes in peripheral tissue oxygenation (StO2) and hemoglobin content (THI) consequent to central hypovolemia, 4) forearm NIRS measurements are more sensitive to LBNP-induced hypovolemia than thenar NIRS measurements, 5) the sensitivity of these NIRS measurements does not depend on NIRS probing depth, and 6) the LBNP-induced shift of blood volume from the upper body to the lower body is reflected by the decreased THI in the forearm and an increased THI in the calf.

To compensate for the central hypovolemia induced by LBNP, both HR and SVR increased. Initially, at mild central hypovolemia (i.e., SV=100-80 % of bsln), SVR was increased approximately by 10 % while HR remained unaffected. Then, during further reduction of SV (i.e., SV=<80 % of bsln), HR increased with decreasing SV while SVR remained elevated around 10 % above baseline level. Hence, here we show that compensation for mild hypovolemia is a biphasic response in which the SVR immediately increases followed by an increase in HR. As HR does not respond immediately to a reduction in SV and SVR cannot compensate during further reduction of SV, these parameters are suboptimal for monitoring the onset and progression of hypovolemia.

In general, the increased HR and SVR compensated adequately for the decreased SV and maintained CO and MAP around baseline level during the entire LBNP protocol. It must be noted, however, that cardiovascular collapse is not included in the analysis since the aim of the present study was to integratively investigate the sensitivity of NIRS to reflect the degree of (compensated) hypovolemia during LBNP. Thus, in this early stage of hypovolemia, CO and MAP were maintained near baseline level, but by definition, at the moment of collapse, both CO and MAP rapidly decreased (data not

shown). However, this study focuses on the early detection of hypovolemia, i.e., when hypovolemia is still adequately compensated.

Earlier studies have demonstrated that during LBNP blood flow in the upper body peripheral regions decreases due to vasoconstriction induced by augmented muscle sympathetic nerve activity [30] and cardiopulmonary receptor unloading [31]. This is supported by our measurement of increased SVR during LBNP. Hansen et al. and Hachiya et al. have shown that this LBNP-induced (upper) body peripheral vasoconstriction is reflected by forearm NIRS measurements using a 30 mm NIRS probe size [9, 32]. Moreover, Fadel et al. showed that during LBNP forearm NIRS measurements using a 20 mm NIRS probe size highly correlated with forearm blood flow velocity measured by Doppler ultrasound [33]. Reduced forearm blood flow and reduced THI result in greater microcirculatory oxygen extraction and thereby reduced forearm StO2 [34]. In summary, it is well-established that central hypovolemia leads to (upper) body peripheral vasoconstriction and that hypovolemia reflected by forearm NIRS measurements. Therefore, NIRS might be suited to monitor the volume status of intensive care patients or (post)surgical patients.

In previous studies, NIRS was used to identify hypoperfusion, predict organ dysfunction, and to guide resuscitation in trauma patients [35]. Crookes et al. showed that thenar StO2 could reflect severe hypovolemic shock, but could not identify mild or moderate shock [36]. Cohn et al. reported no differences in thenar StO2 values between trauma patients and healthy volunteers. For this reason, NIRS cannot identify hypovolemia directly by assessment of steady-state (or baseline) StO2. However, these measurements were all carried out on thenar and no THI values were reported [35-36].

Previously, we have found that forearm StO2 is a more sensitive parameter for detecting hypovolemia-induced peripheral vasoconstriction compared to thenar StO2 [11]. This LBNP study confirms and strengthens our earlier findings. First, in a pilot study, we found that forearm StO2 was more responsive to head-up tilt compared to thenar StO2 [12]. Second, in a simple study on multi-depth thenar and forearm StO2 before and after a posture change, it was shown that forearm StO2 was more responsive to the hemodynamic changes associated with the posture change compared to thenar StO2, independent of NIRS probing depth, which confirmed the findings of the pilot study [11]. Here we measured StO2 and THI with two multi-depth NIRS devices on the forearm and thenar simultaneously and again found that forearm StO2 and THI were more sensitive to central hypovolemia compared to thenar StO2 and THI. Although the marginal decrease (2-3 %) from baseline to SV=60-40 % of bsln in thenar StO2 is statistically significant, this finding is not (clinically) relevant. Forearm StO2, on the other hand, did decrease notably (8-9 %) and approximated the decrease in forearm StO2 observed previously (11% at a SV=~40 %) [7,8]. Hence, the thenar and forearm NIRS measurements in the present study are comparable to those in [7,8], even though different NIRS devices were used. However, as this is the first study measuring StO2 and THI simultaneously in the thenar and forearm at multiple depths, we have now established that the sensitivity of the NIRS measurements is not dependent on probing depth but rather depends on measurement site. This finding

rebuts the hypothesis postulated previously that the 15 mm probe size on the forearm would be too small to reflect hypovolemia [8].

A potential physiological explanation for the difference between the thenar and forearm in terms of sensitivity to hypovolemia is that the circulation in the hands might be well-preserved during cardiovascular challenges. An example of such a phenomenon is cold-induced vasodilation during severe hypothermia, which opens the (micro)circulation to the hands in order to prevent cold-induced necrosis and thereby increase the chances of survival [37]. However, whether such a response also exists in case of hypovolemia remains unknown. Either way, although in the present study we could not identify why the forearm microcirculation is more sensitive to changes in volume status compared to the thenar microcirculation, we have clearly shown that this is indeed the case and that the forearm is a more appropriate measurement site for NIRS for monitoring changes in the peripheral microcirculation in response to changes in volume status.

It should be noted, however, that there are some practical considerations regarding the use of NIRS in critically ill patients and the extrapolation of the results we present here to clinical scenarios. First, in the present study, no severe hypovolemia with hypotension or shock was included. However, the main objective was to use NIRS to detect hypovolemia in the compensated phase (i.e., with maintained blood pressure) and to the test the hypothesis that the sensitivity of NIRS to reflect the degree of (compensated) hypovolemia would be affected by the application site and probing depth. This we have clearly shown, as differences between NIRS probing sites could be detected in this early, compensated phase of central hypovolemia. Second, it is important to realize that the NIRS measurements reflect changes in the peripheral microcirculatory perfusion and that in the present study, these changes were induced by application of LBNP. We anticipate that vasoactive drug-related changes in peripheral microcirculatory perfusion would also be detected. However, when patients are on vasoactive drugs that prevent the peripheral microcirculation to respond to changes in their volume status, NIRS would obviously not be able to detect hypovolemia as no changes would occur in the peripheral microcirculation. Third, another aspect potentially affecting the detection of hypovolemia using NIRS is peripheral edema. During LBNP, no peripheral edema was present. Still, the LBNP-related central hypovolemia is comparable to fluid loss-related hypovolemia as this type of hypovolemia would also decrease stroke volume and lead to the activation of compensatory mechanisms such as increased heart rate and systemic vascular resistance. Whether the NIRS measurements would be able to reflect the degree of hypovolemia in the presence of peripheral edema, however, remains to be established as tissue edema limits the oxygen offloading in the microcirculation and might thereby keep StO2 artificially high. The THI, in contrast, would probably decrease in case of fluid loss-related hypovolemia as the THI is directly related to peripheral vascular tone. Fourth, other approaches, such as the assessment of stroke volume variations (SVV) or pulse pressure variations (PPV), might also provide information on changes in volume status. However, SVV and PPV both focus on the hypovolemia-related changes that occur on the arterial side of the circulation and therefore, in contrast to NIRS, do not

reflect changes in the adequacy of tissue oxygenation that occur due to hypovolemia. Fifth, and final, the changes in observed in StO2 and THI are rather small (i.e., ~8-9 %) and might be difficult to detect in clinical practice. Therefore, whether the sensitivity of NIRS to detect hypovolemia by could be increased by addition of a dynamic test, such as a vascular occlusion test, should be explored [21].

In conclusion, the application of LBNP results in a significant blood volume shift and SV reduction that is countered by an increase in SVR and HR, which adequately compensate for the decrease in SV and the maintenance of CO and MAP near baseline level. NIRS can be used to detect central hypovolemia and applied on the forearm, NIRS is more sensitive to reflect changes in SV than when applied on the thenar. The sensitivity of NIRS does not depend on the NIRS probing depth. The present study confirms the first part of the hypothesis that application site would affect the sensitivity of NIRS and rejects the second part of the hypothesis that probing depth would affect the sensitivity of NIRS.

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V

RED BLOOD CELL TRANSFUSIONS AND TISSUE OXYGENATION IN ANEMIC HEMATOLOGY OUTPATIENTS

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ABSTRACT

There is little clinical evidence that red blood cell (RBC) transfusions improve oxygen availability at the microcirculatory level. We tested the hypotheses that anemia in chronically anemic patients with relatively healthy microcirculation would be associated with low tissue hemoglobin (Hb) and tissue oxygenation levels and that these conditions would be improved after RBC transfusions.

Near-infrared spectroscopy (NIRS) was used to determine tissue oxygen saturation (StO2) and tissue Hb index (THI; an index of the amount of Hb in the NIRS measurement volume) in the thenar eminence and sublingual tissue before and 30 minutes after RBC transfusions in 20 chronically anemic hematology outpatients. Data are presented as median (25 % -75 %).

The patients received three (two to three) bags of RBCs in saline-adenine-glucose-mannitol with an age of 21 (7-21) days, which was infused intravenously at the rate of 0.7 bag/hr. RBC transfusions significantly increased hematocrit level from 26 % (24-28) to 32 % (30-34; $p<0.0001$), Hb level from 8.2 (7.6-8.9) g/dL to 11.0 (9.9-11.8) g/dL ($p<0.0001$), whole blood viscosity from 3.4 (3.1-3.5) mPa/sec to 4.2 (4.0-4.5) mPa/sec ($p<0.0001$), thenar StO2 from 81 % (80-84) to 86 % (81-89; $p=0.002$), thenar THI from 11.2 (9.3-13.3) AU to 13.7 (9.7-15.3) AU ($p=0.024$), sublingual StO2 from 86 % (81-89) to 91 % (86-92; $p<0.0001$), and sublingual THI from 15.2 (13.0-17.4) AU to 17.2 (13.5-19.7) AU ($p=0.040$).

We conclude that RBC transfusions were successful in improving these variables, although anemia in chronically anemic hematology outpatients was not associated with low StO2 and THI levels,

INTRODUCTION

The primary goal of red blood cell (RBC) transfusions is to increase hematocrit (Hct) and blood hemoglobin (Hb) levels, thereby improving microcirculatory Hb availability and, ultimately, tissue oxygenation. To date, only a few clinical studies have investigated the effects of RBC transfusions on peripheral microcirculation. In a study by Sakr and colleagues [1] in septic patients, no changes were found after RBC transfusions in microcirculatory density and perfusion when measured sublingually using orthogonal polarization spectral imaging. Similarly, Creteur and colleagues found no effect of RBC transfusions on microcirculatory oxygenation in septic and nonseptic intensive care patients, as measured in the thenar eminence using near-infrared spectroscopy (NIRS) [2]. Contrary to these studies, we recently demonstrated improved microcirculation upon RBC transfusion in cardiac surgery patients [3]. The contrasting results of these studies may be related either to the impaired microcirculation in septic patients compared with cardiac surgery patients or to the fact that intensive care patients are chronically (or progressively) anemic, while cardiac surgery patients suffer from a more acute onset of anemia [4]. In this respect, it has been shown that chronic anemia leads to the development of compensatory mechanisms, such as an increased release of oxygen, due to higher levels of 2,3-diphosphoglycerate acid [5]. In this study, we focused on the latter factor and investigated the effects of RBC transfusions on microcirculatory oxygenation and Hb availability in anemic hematology outpatients, a chronically anemic patient group with relatively healthy microcirculation (compared with that of sepsis patients). We tested the hypotheses that anemia in these patients would be associated with low levels of tissue oxygen saturation (StO2) and a low tissue Hb index (THI) and that these conditions would improve after RBC transfusions. The THI reflects the amount of Hb in the NIRS measurement volume. This variable depends on both the systemic Hb level, which is low because these patients are anemic, and the peripheral vascular tone (i.e., vasoconstriction decreases THI and vasodilation increases THI). In this light, we expected that, as with the systemic Hb level, THI would be low in anemic patients. Because StO2 reflects mainly microcirculatory oxygenation, this variable indicates the balance between microcirculatory oxygen delivery and tissue oxygen consumption. Hypothesizing that these patients would have a low THI, we also expected that the StO2 would be low. In this study, we aimed to identify whether the chronic nature of anemia is the limiting factor in the efficacy of RBC transfusions in some anemic patient groups.

MATERIAL AND METHODS

This study protocol was approved by the institutional medical ethics committee of the AcademicMedical Center of the University of Amsterdam. Written informed consent was obtained from all participating patients. This study was performed in compliance with the principles established in the Helsinki Declaration.

Patients

Anemic hematology outpatients requiring RBC transfusions were considered eligible for participation in this study and were recruited during a 2-month period. The threshold for transfusion was set at a Hb level of less than 9.6 g/dL according to standard clinical practice in the Department of Clinical Hematology of the Academic Medical Center and Dutch transfusion guidelines (CBO guidelines 2011, p. 117) [6]. Transfusion was stopped after reaching this threshold or after infusion of 3 RBC units. RBC units were prepared according to the standards of Sanquin, the Netherlands national blood bank. The units contained concentrated RBCs obtained after centrifuging whole blood to remove the buffy coat, adding saline-adenine-glucose-mannitol solution, and filtering out the white blood cells (WBCs) [6,7]. Upon arrival at the short-stay center for RBC transfusions and after a cross-match blood examination, all participants were subjected to the same investigational procedures, that is, standard determination of venous blood (gas) and hemodynamic variables and NIRS measurements that were performed sublingually and in the thenar eminence, as described below.

NIRS

The patients were placed in a semi supine (30° head-up) position in a hospital bed with their hands (palm up) and arms passively maintained at heart level. The arm opposite to the infusion arm was used in the NIRS measurements. A NIRS device (InSpectra 650, Hutchinson Technology, Hutchinson, MN) equipped with a 15-mm probe was used to measure StO2 (%) and THI (arbitrary units [AU]) noninvasively before and 30 minutes after blood transfusion in the thenar eminence, as previously described [3,8]. For the sublingual NIRS measurements, an identical probe was used, which was placed on the sublingual mucosa parallel to the frenulum linguae on the same side as the thenar measurements. The spectrometer uses a reflectance mode probe with a 1.5-mm optical fiber to illuminate tissue and a 0.4-mm optical fiber, which is spaced 15 mm from the illuminating fiber, to detect backscattered light. The NIRS measurement depth is estimated as approximately half of the distance between the illumination and detection fibers [9-11]. Both the StO2 and the THI calculations from the NIRS signal have been validated by Myers and colleagues [9,12].

Whole blood viscosity assessment

Whole blood was collected in sterile blood tubes, each of which contained 10 mL of blood and 18 mg of K2EDTA, as an anticoagulant. A Couette low-shear viscometer (Contraves LS-30, proRheo GmbH, Althengstett, Germany) was used to measure whole blood viscosity at shear rates of 0.87, 2.19, 5.49, 10.15, 47.1, and 87/sec, in decreasing order [13]. All measurements were performed at a stable temperature of 37°C and were completed within 1 hour of the time that the blood sample was collected.

Statistical analysis

Statistical analysis was performed using computer software (Prism 5.0, GraphPad Software, La Jolla, CA). The Wilcoxon matched-pairs test was used for comparative

analysis of data sets obtained before and after RBC transfusion. All data are presented as median values followed by the 25 % to 75 % range in parentheses. Differences were considered significant at $p<0.05$.

RESULTS

Twenty consecutive anemic clinic outpatients (11 males and 9 females) with various hematological malignancies with an age of 65 (60-68, Table 1) years requiring RBC transfusions participated in this study. The patients received 3 (2-3) bags of packed red blood cells with an age of 21 (7-21) days, which were infused intravenously at the rate of 0.7 bags/h.

Table 1: Patient characteristics. Data are presented in median (25^{th}-75^{th}) unless indicated otherwise.

Demographics	
Age	65 (60-68) years
Sex (male:female)	11: 9
Underlying disease	
Myelodysplastic Syndrome	7 patients
Myelofibrosis	4 patients
Multiple Myeloma	3 patients
Acute Myeloid Leukemia	2 patients
Chronic Myeloid Leukemia	1 patient
Chronic Lymphocytic Leukemia	1 patient
Metastasis bleeding	1 patient
Non-Hodgkin Lymphoma	1 patient
Transfusion characteristics	
Number of RBC	3 (2-3) bags
Age of transfused blood	21 (7-21) days

All NIRS measurements were performed successfully and without any inconvenience or discomfort to the patients. Hct, Hb, whole blood viscosity, and hemodynamic variables before and 30 minutes after RBC transfusions are presented in Table 2.

RBC transfusions increased the Hct from 26 % (24-28) to 32 % (30-35; $p<0.0001$), Hb from 8.2 (7.6-8.9) g/dL to 11.0 (9.8-11.8) g/dL ($p<0.0001$; Fig. 1A), and whole blood viscosity from 3.4 (3.1-3.5) mPa/sec to 4.2 (4.0-4.5) mPa/sec ($p<0.0001$). Mean arterial pressure (MAP) increased from 84 (77-92) mmHg to 94 (84-107) mmHg ($p=0.006$), and heart rate decreased slightly from 82 (71-91) bpm to 78 (71-87) bpm ($p=0.088$; Fig. 1B).

Table 2: Blood and hemodynamic parameters

		Before transfusion			After transfusion				
Parameter	Unit	Median	25th	75th	Median	25th	75th	p-value	Normal range
Hematocrit	[%]	26	24	28	32	30	35	<0.0001	36 – 51
Hemoglobin	[g/dL]	8.2	7.6	8.9	11.0	9.8	11.8	<0.0001	12.1 – 17.7
Blood viscosity	[mPa·s]	3.4	3.1	3.5	4.2	4.0	4.5	<0.0001	3.0 – 4.0
Systolic BP	[mmHg]	135	116	147	145	120	158	0.067	90 – 140
Diastolic BP	[mmHg]	72	61	80	77	73	85	0.009	60 – 80
MAP	[mmHg]	84	77	91	94	84	107	0.006	70 – 90
Heart rate	[bpm]	82	71	91	78	71	87	0.088	60 – 90
Temperature	[°C]	36.9	37.7	37.1	37.1	36.9	37.6	0.008	35.5 – 37.5

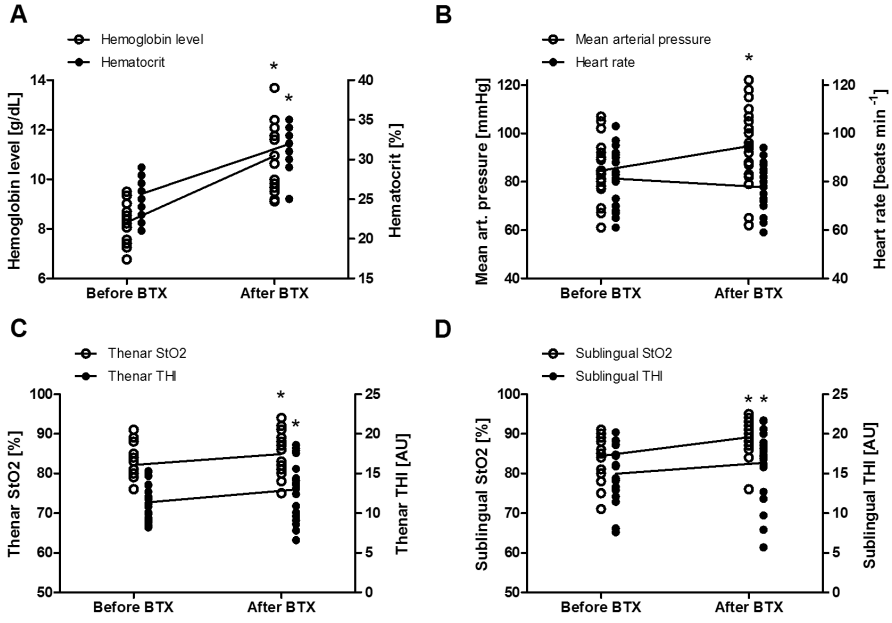

Figure 1. A) Hemoglobin (Hb; g/dL) and hematocrit (Hct; %) levels before and after transfusion, **B)** mean arterial pressure (MAP; mmHg) and heart rate (HR; beats per minute) before and after transfusion, **C)** thenar tissue oxygenation (StO2; %) and tissue hemoglobin index (THI; AU) before and after transfusion, and **D)** sublingual tissue oxygenation (StO2; %) and tissue hemoglobin index (THI; AU) before and after transfusion. BTX=blood transfusion; *indicates p<0.05 versus before BTX.

After the RBC transfusion, the thenar (Fig. 1C) and sublingual (Fig. 1D) StO2 and THI increased significantly. The thenar StO2 and THI increased from 81 % (80-84) to 86 % (81-89; p=0.002) and from 11.2 (9.3-13.3) AU to 13.7 (9.7-15.3) AU (p=0.024), respectively. The sublingual StO2 and THI increased from 86 % (81-89) to 91 % (86-92; p<0.0001) and from 15.2 (13.0-17.4) AU to 17.2 (13.5-19.7) AU (p=0.040), respectively.

DISCUSSION

In this study, we investigated the effects of RBC transfusions on microcirculatory oxygenation in chronically anemic patients with relatively healthy microcirculation. We hypothesized that anemia in these patients would be associated with low StO2 and Hb availability (THI), which would increase after the RBC transfusion. This is an important issue because studies addressing the efficacy of RBC transfusion at the microcirculatory level have only concerned critically ill patients and showed little or no effect of RBC transfusions on microcirculation [1,2]. In this study, we demonstrate that, although anemia in chronically anemic outpatients was not associated with low StO2 and THI levels, RBC transfusions were successful in improving these conditions.

Although RBC transfusions successfully improved such conditions as Hct and Hb levels, whole blood viscosity, and MAP [2,3,14,15], the efficacy of RBC transfusions in improving microcirculation and, ultimately, tissue oxygenation is poorly understood. Recently, microcirculation has gained increasing attention as an independently functioning physiological compartment in which the roles of the glycocalyx, RBCs, and WBCs are shifted from passive components to active mediators [2,3].

Blood transfusion practice and policies have been a topic of debate since several large clinical studies demonstrated the potentially harmful effects of allogeneic blood transfusions, such as infection transmission, immunosuppression, inflammation and coagulation in the lung, and atrial fibrillation [16-19]. These studies concluded that restrictive transfusion thresholds and regimens contribute to better clinical outcomes. Whether the adverse effects of blood transfusions are due to the composition of the transfused blood (e.g., leukoreduced vs. leukodepleted blood) or other factors (e.g., age of the transfused blood) remains elusive. Nevertheless, the decision of whether to give a blood transfusion remains a consideration of the beneficial effects of transfusion, for example, increasing the systemic oxygen-carrying capacity, microcirculation, and ultimately, tissue oxygenation versus the adverse effects of transfusion. In this line of reasoning, blood transfusion not only improves systemic Hct but also increases whole blood viscosity, as we have shown here. This increase in viscosity, in turn, stimulates microvascular perfusion as a result of shear stress-induced vasodilation, as described by Lenz and colleagues [20].

To date, only a few studies have investigated the direct effects of RBC transfusions on peripheral microcirculation [1-3,21], and only two studies have shown a beneficial effect of RBC transfusions on microcirculation in adults [3] and anemic preterm infants [21]. The contrasting results of these studies might be explained by the studied patient populations because the studies showing no effect of RBC transfusions were carried out in (septic) intensive care patients in whom the microcirculation is significantly

impaired with endothelial dysfunction and abnormal endogenous RBCs [1,2,4,22]. This microcirculatory dysfunction is much less prevalent in surgical patients [3] and preterm infants [21] possibly explaining the discrepancy between the different studies.

In addition to differences in the microcirculation caused by systemic inflammatory responses, the chronic nature of anemia also may limit the efficacy of RBC transfusions on microcirculatory oxygenation. Therefore, we studied the effects of RBC transfusions on microcirculatory oxygenation in anemic outpatients with a hematologic malignancy but with relatively healthy microcirculation (compared with that of sepsis patients). We showed that the microcirculatory oxygenation and Hb availability were slightly but significantly increased after RBC transfusions. Although the THI and StO2 increases were modest, this was a highly consistent finding. The sensitivity of both variables to anemia and blood transfusion has been questioned [2]. Here we show that blood transfusion did significantly improve StO2 and THI, but whether the small changes in THI or StO2 are of any clinical significance remains to be established. In intensive care patients, blood transfusion did not improve these NIRS variables. Thus, in this study, we have found that there is a difference in response to blood transfusion between intensive care patients and hematology outpatients. However, the mechanisms preventing blood transfusions from effectively reaching the microcirculation in intensive care patients are yet unknown.

This study reports on the use of NIRS on multiple locations to examine the effect of RBC transfusions and introduces the sublingual site for NIRS measurements. Although multisite NIRS measurements have been described before [8,23,24], analysis of sublingual tissue to examine the effects of RBC transfusions on microcirculation was not performed previously. A study by Yuruk and coworkers [3] applied a spectroscopic technique similar to the NIRS technique used here; however, due to the different wavelength range and probe spacing used in their study, a much smaller measurement volume was captured. Furthermore, to our knowledge, THI has not been used to describe the effects of RBC transfusions at the microcirculatory level. Because THI reflects microcirculatory oxygen-carrying capacity in tissue, this variable is important in studying the effects of RBC transfusions. Previously, NIRS has been employed to identify hypoperfusion and to guide resuscitation in trauma patients [25,26]. Cohn and coworkers reported no differences in StO2 measurements in the thenar between trauma patients and healthy volunteers [25]. Crookes and coworkers showed that thenar StO2 values could reflect severe hypovolemic shock, but could not identify mild or moderate shock [26]. Furthermore, studies in healthy volunteers have also shown that StO2 and THI respond poorly to changes in volume status [23,27]. In one study, 500 mL of blood each was donated by healthy volunteers, and no significant changes in StO2 were observed [27]. In another study on healthy volunteers subjected to lower-body negative pressure (a model in which application of a vacuum to the lower body shifts blood from the upper to the lower body, creating central hypovolemia), StO2 and THI levels were shown to decrease only slightly [23]. Altogether, it seems that moderate changes in patient volume changes are poorly reflected by peripherally measured StO2 and THI levels.

Although the development of NIRS has recently opened the field of bedside monitoring of tissue oxygenation (StO2) and Hb content (THI) at the microcirculatory

level in a variety of patient populations, the interpretation of the measurements should be done with great care. NIRS has been used for the detection of sepsis and as a predictor of patient outcomes [28-30]. NIRS has, moreover, been employed in trauma patients to identify tissue hypoperfusion and to guide resuscitation [25,26]. However, the relative contribution of the arterioles, capillaries, and venules to the NIRS measurement is unknown, which impedes the full physiological interpretation of the StO2 and THI values in separate microvascular compartments. Furthermore, the NIRS technology is not able to measure flow, which further limits the interpretation of the measurements because flow is a major determinant of tissue oxygenation. Another important consideration is the interpretation of THI, which does not reflect only systemic Hct and Hb levels but also depends on the peripheral (micro)vascular tone [31]. Nonetheless, although baseline StO2 and THI levels in our anemic outpatients were within the normal range [8,23], the values associated with both variables increased after RBC transfusions, indicating that transfusions can effectively improve microcirculatory oxygenation in outpatients with hematologic malignancies.

A possible limitation of our study may be that we selected anemic outpatients with various hematologic malignancies, meaning that we cannot exclude the influence of different forms of therapy before this study, such as erythropoietin and high-dose chemotherapy, which may have affected microcirculation. However, since the baseline microcirculatory oxygenation appeared to be within the normal range and none of the patients was treated with chemotherapy at the time of the study, we consider this effect to be minor. Furthermore, for this study, a relatively high Hb threshold was applied compared with other anemic patient groups. However, according to Dutch guidelines and the transfusion policy of the Department of Clinical Hematology, this relatively old patient group receives RBC transfusions at an Hb level of less than 9.6 g/dL [6]. Although the threshold of 9.6 g/dL was used here, the mean Hb concentration before transfusion in this study was 8.2 g/dL, which is considerably lower. In contrast with thresholds for platelet transfusions, there are no prospective comparative trials that have established the optimal Hb concentration in this specific patient group. However, the transfusions have been carried out according to current clinical standards in the Netherlands and, therefore, we chose to use this threshold. We expect that the slight (but highly consistent) increase in StO2 and THI levels observed in this study will be more extensive when a lower transfusion threshold is applied.

In conclusion, we demonstrated that, although anemia in chronically anemic hematology outpatients was not associated with low StO2 and THI levels, RBC transfusions were successful in increasing the values associated with these variables. We also showed that the chronic nature of anemia is not the limiting factor in the efficacy of RBC transfusions at the microcirculatory level in some anemic patient groups.

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SUMMARY AND CONCLUSIONS

Throughout history, blood circulation in the human body has drawn a lot of attention, which was further encouraged with the observations and publications of William Harvey, Marcello Malpighi, and James Blundell. Technological advancements led to new discoveries and enabled the development of monitoring tools. As part of the circulation, the microcirculation is the most important system for the exchange of oxygen, carbon dioxide, and nutrients. The microcirculation comprises blood vessels with a diameter < 100 μm, i.e. arterioles, capillaries, and venules. Monitoring the microcirculation has demonstrated to provide valuable prognostic information on outcome of patients. To study the effects of hypovolemia on the microcirculation isolated from the other potentially confounding factors occurring in critically ill patients, we have developed a model in which lower body negative pressure (LBNP) is applied in healthy volunteers to create central hypovolemia, which allows elaborate microcirculatory studies in which volume status can be modulated in a controlled fashion.

LBNP-induced progressive hypovolemia is associated with a decrease in stroke volume (SV) triggering compensatory mechanisms and therefore, reliable continuous measurements of SV are of key importance to assess the effects of application of LBNP. When SV decreases, compensatory mechanisms, such as increased heart rate (HR) and systemic vascular resistance (SVR) will maintain cardiac output (CO). In Chapter I continuous beat-to-beat contour analysis (Nexfin) was compared with inert gas rebreathing and respired gas analysis. Nineteen healthy male volunteers, who completed a medical questionnaire, were subjected to bicycle ergometry testing with increasing workloads. Cardiac output, HR, and SV were determined using Nexfin, inert gas rebreathing, and respired gas analysis. Rebreathing tests were performed at baseline and at HR = 150 bpm, while Nexfin results were recorded continuously. Respired gas analysis was performed in a maximal exercise protocol in a subgroup. The CO values derived from the Nexfin- and inert gas rebreathing methods were well correlated and the limits of agreement were 30.3 %. Nexfin- and respired gas analysis-derived CO values correlated even better. At rest, the rebreathing maneuver increased HR by 13 bpm, SV remained unaffected, while CO increased by 1.0 L/min. Rebreathing did not affect these parameters during exercise. In conclusion, Nexfin continuous beat-to-beat pulse contour analysis is an appropriate method for noninvasive assessment of CO during exercise.

Increasing SVR early in the onset of hypovolemia results in reduced peripheral perfusion. To investigate the ability of the pulse oximeter-derived Peripheral Perfusion Index (PPI) to reflect early peripheral vasoconstriction during progressive hypovolemia, we applied lower body negative pressure (LBNP) in healthy volunteers until onset of cardiovascular collapse in Chapter II. Twenty-five healthy volunteers were subjected to a stepwise LBNP protocol and SV, heart rate, blood pressure, SVR, and CO were recorded using volume clamp finger plethysmography. The PPI assessment was done by pulse oximetry. During the first LBNP step, SV decreased, while heart rate remained unaltered. In contrast, SVR increased and PPI decreased, reflecting vasoconstriction and decreased peripheral flow. During further progression of LBNP, both SV and HR changed proportionally to the applied negative pressure, whereas SVR and PPI remained unaltered around the LBNP=-20 mmHg level throughout the rest of the protocol. As

a result of adequately functioning compensatory mechanisms, BP and CO did not change significantly throughout the experiment. In conclusion, LBNP results in a decrease in SV and an increase in HR and SVR. The latter two can be successfully monitored using the applied pulse oximeter and the PPI can be used as a complementary hemodynamic monitoring technique for the early detection of central hypovolemia.

In Chapter III we investigated the microcirculatory alterations during hypovolemia in more detail. The objective of the study described in this chapter was to test the hypothesis that controlled, adequately compensated, central hypovolemia in subjects with intact autoregulation would be associated with decreased peripheral microcirculatory diffusion and convection properties and, consequently, decreased tissue oxygen carrying capacity and tissue oxygenation. Furthermore, we evaluated the impact of hypovolemia-induced microcirculatory alterations on resting tissue oxygen consumption. To this end, twenty-four subjects were subjected to a progressive LBNP protocol of which fourteen reached the end of the protocol. At baseline and at LBNP=-60 mmHg, sidestream dark field images of the sublingual microcirculation were acquired to measure microvascular density and perfusion. Near-infrared spectroscopy (NIRS) was used to measure thenar and forearm tissue oxygenation (StO2) and hemoglobin content (THI) and a vascular occlusion test (VOT) was performed to assess resting tissue oxygen consumption rate. Application of LBNP resulted in a significantly decreased microvascular density (oxygen diffusion) and perfusion (oxygen convection), significantly decreased THI and StO2 parameters, and an unaltered resting tissue oxygen consumption rate. In conclusion, SDF imaging in combination with NIRS is associated with decreased microcirculatory diffusion and convection properties and, consequently, decreased tissue oxygen carrying capacity (THI) and tissue oxygenation (StO2) in subjects with intact autoregulation during controlled, adequately compensated, central hypovolemia. Furthermore, using a VOT we found that resting tissue oxygen consumption remained unaltered under conditions of adequately compensated central hypovolemia.

We further explored the use of NIRS during progressive hypovolemia by testing the hypothesis that the sensitivity to reflect the degree of (compensated) hypovolemia is affected by the NIRS application site and probing depth in Chapter IV. Therefore, we simultaneously applied multi-site (thenar and forearm) and multi-depth NIRS in LBNP. Twenty-four healthy male volunteers were subjected to a LBNP protocol and SV and HR were measured using volume-clamp finger plethysmography. The StO2 and THI results of the thenar and the forearm were compared and the shift of blood volume towards the lower extremities was monitored by calf THI and the thoracic fluid content (TFC; bioreactance monitoring). Application of LBNP resulted in a significant reduction in stroke volume which was accompanied by a reduction in forearm StO2 and THI. Furthermore, calf THI increased and TFC decreased, which confirmed the occurrence of hypovolemia due to a shift of fluid to the lower body, which was compensated by an increased HR and SVR. In summary, NIRS can be used to detect changes in StO2 and THI consequent to central hypovolemia. Forearm NIRS measurements are more sensitive to hypovolemia than thenar NIRS measurements, verifying the first part of the hypothesis. The sensitivity of NIRS measurements does not depend on NIRS

probing depth, rejecting the second part of the hypothesis. The LBNP-induced shift of blood volume is demonstrated by the decreased THI in the forearm and an increased THI in the calf.

In Chapter V we studied the clinical applicability of NIRS and translated the findings of previous studies into clinical practice. Little clinical evidence is available that red blood cell (RBC) transfusions improve oxygen availability at the microcirculatory level. We tested the hypotheses that anemia in chronically anemic patients with a relatively healthy microcirculation would be associated with low tissue hemoglobin and tissue oxygenation levels and that these conditions would be improved following RBC transfusions. Near-infrared spectroscopy was used to determine StO2 and THI in the thenar eminence and sublingual tissue prior to and 30 minutes after RBC transfusions in twenty chronically anemic hematology outpatients. RBC transfusions significantly increased hematocrit, hemoglobin, and whole blood viscosity levels. Thenar and sublingual StO2 and THI were not decreased at the baseline measurements, but increased after RBC transfusion. We conclude that anemia in chronically anemic hematology outpatients was not associated with low StO2 and THI levels, yet RBC transfusions were successful in improving these parameters.

The research described in this thesis demonstrates the ability of currently available techniques to monitor microcirculatory alterations in subjects with hypovolemia and anemia. Microcirculatory parameters showed (early) alterations in response to hypovolemia, but appeared within the normal range in chronic anemic outpatients. Further research is required to use the described modalities to guide microcirculatory-targeted therapies.

SAMENVATTING EN CONCLUSIES

De bloedcirculatie in het menselijk lichaam heeft in de loop van de geschiedenis veel aandacht gehad. Deze belangstelling werd aangewakkerd door de observaties en publicaties van William Harvey, Marcello Malpighi en James Blundell. Technologische innovaties leidden tot nieuwe inzichten en maakten de ontwikkeling van meetinstrumenten mogelijk. Als onderdeel van de bloedcirculatie is de microcirculatie het belangrijkste onderdeel voor de uitwisseling van zuurstof, koolstofdioxide en voedingsstoffen. De microcirculatie bestaat uit vaten met een diameter kleiner dan 100 µm: de arteriolen, venulen en capillairen. Het is reeds aangetoond dat observatie van de microcirculatie waardevolle informatie over de prognose en klinische uitkomst van patiënten kan verschaffen. Om de effecten van hypovolemie op de microcirculatie zonder vertroebelende factoren te bestuderen, is er een model ontwikkeld waarin lower body negative pressure (LBNP) op gezonde vrijwilligers wordt toegepast. Op deze manier wordt centrale hypovolemie gecreëerd en kunnen microcirculaire studies naar volume-veranderingen op een gecontroleerde manier worden verricht.

De hypovolemie die door LBNP wordt geïnduceerd gaat gepaard met een afname in het slagvolume en dit leidt weer tot compensatiemechanismen, zoals een verhoogde hartslag en toegenomen vasculaire weerstand. De aanpassingen leiden ertoe dat de hoeveelheid bloed die per minuut het hart uitgepompt wordt, constant blijft. In Hoofdstuk I is de continue pulse contour analyse (Nexfin) met inert gas rebreathing en een ventilatie analyse methode vergeleken. Negentien gezonde mannelijke vrijwilligers hebben een medische vragenlijst ingevuld en een fietstest gedaan. Hartslag, slagvolume en hartminuutvolume werden met behulp van Nexfin, inert gas rebreathing en de ventilatie analyse methode gemeten. Rebreathing metingen werden voor de start van het protocol gedaan en bij een hartslag 150 bpm. De Nexfin waarden werden continu gemeten. De ventilatie analyse methode werd in het kader van een ander protocol uitgevoerd, waarin een subgroep van de gezonde vrijwilligers tot maximale inspanning ging en zowel de Nexfin resultaten als de waarden van de ventilatie analyse methode continu geregistreerd werden. De resultaten van Nexfin en inert gas rebreathing correleerden goed met elkaar en de grenzen van overeenkomst (limits of agreement) waren 30,3 %. Nexfin en de ventilatie analyse methode correleerden nog beter. De rebreathing methode bleek de hartslag met 13 bpm te verhogen, het hartminuutvolume met 1,0 L/min, maar veranderde het slagvolume niet. Tijdens inspanning bleven deze parameters ongewijzigd. We concluderen dat Nexfin pulse contour analyse een geschikte methode is voor het noninvasief meten van hartminuutvolume tijdens inspanning.

In Hoofdstuk II hebben we LBNP toegepast als model voor centrale hypovolemie om de geschiktheid van de Peripheral Perfusion Index (PPI) te bepalen voor het vroegtijdig detecteren van vasoconstrictie tijdens (progressieve) hypovolemie bij vrijwilligers. Tijdens het LBNP protocol werden slagvolume, hartslag, bloeddruk en hartminuutvolume gemeten met volume clamp plethysmografie. De PPI werd bepaald met behulp van een puls oximeter. Tijdens de eerste LBNP stap nam het slagvolume af, maar veranderde de hartslagfrequentie niet. De vasculaire weerstand en de PPI veranderden wel en zijn een indicatie voor vasoconstrictie en afgenomen bloedcirculatie. Tijdens verdere LBNP blootstelling veranderden het slagvolume en

de hartslag proportioneel met de negatieve druk, maar de vasculaire weerstand en PPI bleven op hetzelfde niveau als bij de inductie van LBNP. De bloeddruk en het hartminuutvolume bleven door adequaat functionerende compensatie mechanismen ongewijzigd. Concluderend leidt het LBNP protocol tot een afname in slagvolume en een toename in zowel hartslag als vasculaire weerstand. De hartslag en vasculaire weerstand kunnen met een pulse oximeter gemeten worden en PPI kan als aanvullende hemodynamische observatie-techniek voor de vroege detectie van centrale hypovolemie functioneren.

In Hoofdstuk III zijn de microcirculaire veranderingen tijdens hypovolemie in meer detail onderzocht. Het doel van de studie was om de hypothese te testen dat gecontroleerde, adequaat gecompenseerde, centrale hypovolemie in personen met intacte autoregulatie leidt tot een afname van perifere microcirculaire diffusie en convectie. De verwachting was dat hierdoor de hoeveelheid beschikbare zuurstof in het weefsel en de weefsel-saturatie afgenomen was. Verder hebben we het effect van hypovolemie-geïnduceerde microcirculaire veranderingen op zuurstofconsumptie in weefsel onderzocht. Vierentwintig personen werden aan een LBNP protocol onderworpen, waarvan veertien de laatste stap van dit protocol bereikten. Voor de start van het protocol en bij LBNP=-60 mmHg werden sidestream dark field beelden van de sublinguale microcirculatie gemaakt om de microvasculaire diffusie- en convectieparameters te bepalen. Near-infrared spectroscopy (NIRS) werd in thenar en onderarm toegepast om de weefsel saturatie vast te stellen en er werd een vasculaire occlusie test gedaan om het zuurstofverbruik in rust te bepalen. We hebben gevonden dat LBNP leidt tot significant afgenomen microvasculaire dichtheid (diffusie) en perfusie (convectie), significant afgenomen NIRS waarden en een onveranderde zuurstofconsumptie. Concluderend hebben we aangetoond dat centrale hypovolemie gepaard gaat met afgenomen SDF en NIRS parameters, maar dat de zuurstofconsumptie onveranderd blijft.

Het gebruik van NIRS tijdens toenemende hypovolemie werd in meer detail onderzocht in Hoofdstuk IV. Hierin werd de hypothese getest dat de sensitiviteit om hypovolemie te detecteren door de plek waar de NIRS probe bevestigd wordt en de diepte van het NIRS signaal beïnvloed wordt. Om dit te evalueren zijn er simultaan multi-site (thenar en onderarm) en multi-depth NIRS metingen tijdens LBNP gedaan. Vierentwintig personen participeerden in een LBNP protocol, waarin slagvolume en hartslag met volume clamp plethysmografie gemeten werden. Vervolgens werden de NIRS resultaten met elkaar vergeleken. De verplaatsing van het bloedvolume naar het onderlichaam werd gemonitord met behulp van een NIRS sensor op de kuit en met het meten van de thoraxvochtvolume trend (Thoracic Fluid Content; bioreactance monitoring). De belangrijkste bevinding was dat de afname van NIRS parameters, die in de onderarm het meest sensitief was, niet bepaald werd door de probing depth. Het LBNP model functioneerde adequaat, aangezien het slagvolume afnam, het NIRS signaal op de kuit eveneens veranderde en daarnaast de thoracic fluid content verminderde. Als reactie hierop namen de vasculaire weerstand en de hartslag toe om zo het hartminuutvolume te compenseren. Concluderend kan NIRS gebruikt worden om veranderingen ten gevolge van centrale hypovolemie te detecteren. De metingen

op de onderarm zijn meer sensitief dan de metingen op de thenar en bevestigen het eerste deel van de hypothese. De sensitiviteit wordt echter niet bepaald door de diepte van het NIRS signaal en verwerpt dus het tweede deel van de hypothese.

In Hoofdstuk V is de klinische toepasbaarheid van NIRS bestudeerd en zijn enkele bevindingen uit eerdere hoofdstukken vertaald naar de patiëntenzorg. Er is weinig klinisch bewijs beschikbaar dat bloedtransfusies de beschikbaarheid van zuurstof op microcirculair niveau verbeteren. We hebben de hypothesen getest dat anemie in patiënten met chronische anemie en een relatief gezonde microcirculatie gepaard gaan met relatief lage weefsel hemoglobine en oxygenatie waarden. We verwachtten dat transfusies deze parameters verbeteren. NIRS parameters werden op de thenar en in sublinguaal weefsel zowel voor transfusie als 30 minuten na transfusie gemeten bij 20 hematologie patiënten met chronische anemie. De transfusies leidden tot significant hogere hematocriet-, hemoglobine- en viscositeitwaarden. Zowel thenar als sublinguale NIRS resultaten waren voor transfusie niet verlaagd, maar stegen wel na transfusie. Zodoende concluderen wij dat chronische anemie in hematologie patiënten niet gepaard gaat met lage NIRS parameters, maar dat bloedtransfusies wel succesvol waren om deze parameters te verbeteren.

Het onderzoek beschreven in dit proefschrift laat de mogelijkheden van de huidige beschikbare technieken zien om microcirculaire veranderingen ten gevolge van hypovolemie en anemie te observeren. De microcirculatie demonstreert veranderingen ten gevolge van hypovolemie in een vroege fase, maar met betrekking tot chronisch anemische hematologie patiënten bevonden de resultaten zich binnen normaal waarden. Verder onderzoek is vereist om het gebruik van de onderzochte methoden te kunnen richten op toepassingen bij microcirculatie-geöriënteerde therapieën.

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[#] = *equal contribution*

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CURRICULUM VITAE

Sebastiaan Antonius Bartels was born on the 3rd of November 1982 in Oud en Nieuw Gastel, The Netherlands. In 2001, he obtained the secondary school diploma at the Murmellius Gymnasium in Alkmaar. For one year he attended the Law School from the University of Leiden. In 2002 he started studying Medicine and was M.D. graduated in April 2009. After travelling a couple of months around the world, he started a job at the Department of Translational Physiology of the Academic Medical Center (AMC) of the University of Amsterdam in January 2010, focusing on studying the microcirculation during hypovolemia and anemia under supervision of Prof.dr. Can Ince and Dr. Rick Bezemer. Research activities were expanded to the Department of Intensive Care of the Erasmus Medical Center Rotterdam for evaluation of the microcirculatory effects of blood transfusion, and to the Department of Intensive Care of the Medical Center Alkmaar for research and educational purposes. Prof.dr. Jan Bakker and Prof.dr. Can Ince provided the opportunity for Sebastiaan to obtain his Doctorate of Philosophy at the Erasmus University Rotterdam. In December 2011 he started his training as a clinical chemist at the Sint Lucas Andreas hospital in Amsterdam. Besides clinical research, he enjoys literature, sports, and composing music.

The doors of wisdom are never shut.

Benjamin Franklin

Apart from the known and the unknown, what else is there?

Harold Pinter