Studying the impact of different body positioning, squatting, and unipodal flexion on perfusion in the lower limb – an exploratory approach complemented with optical spectroscopy (TiVi)

Estudo do impacto de diferentes posicionamentos corporais, agachamento e flexão unipodal na perfusão no membro inferior - uma abordagem exploratória complementada pela espectroscopia óptica (TiVi)

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Abstract

Body posture and movement seem to be responsible for multiple variations of microcirculation in the foot, although difficulties associated with measurement during movement have limited their characterization. This pilot study sought to explore a new non-invasive instrument based on polarized light spectroscopy – the TiVi system, under orthostatic and dynamic conditions. Five healthy participants (duly informed, 26.0 ± 6.5 years of age) of both genders were selected. The protocol involved sequential posture changes (orthostatic variation) and dynamic movements (squatting and unipodal flexion). Perfusion variations were evaluated in both feet by laser-Doppler flowmetry (LDF) and polarized spectroscopy (TiVi). Both systems revealed the same type of modification of these flow-related variables. The most pronounced changes were obtained with exercise, especially with squatting, which caused a marked increase in foot perfusion. Less dramatic but in the same direction, the increased perfusion with unipodal flexion was also observed in the contralateral limb. This study confirmed the interest in using the TiVi system in these domains, as well as the appropriateness of this experimental design to look deeper into the impact of lower limb movement on the functional dynamics of the foot.

Keywords: foot microcirculation, LDF, TiVi, orthostatic, symmetrical squatting, unipodal flexion

Resumo

A postura corporal e o movimento parecem ser responsáveis por múltiplas variações da microcirculação no pé, embora mal caracterizadas pelas dificuldades associadas à medição durante o movimento. Este estudo-piloto procurou explorar um novo meio não-invasivo, a espectroscopia de luz polarizada pelo sistema TiVi, em condições ortostáticas e dinâmicas. Foram selecionados cinco participantes (26.0 ± 6.5 anos de idade) saudáveis de ambos os sexos, devidamente informados. O protocolo envolveu modificações sequenciais da postura (variação ortostática) e movimentos dinâmicos (agachamento e flexão unipodal). As variações da perfusão foram avaliadas em ambos os pés através da fluxometria de laser-Doppler (LDF) e da espectroscopia de polarização (TiVi). Ambos os sistemas revelaram o mesmo tipo de modificação das variáveis relacionadas com a alteração de fluxo. Os resultados mais expressivos foram registados com o exercício, em especial com o agachamento provocando um marcado aumento da perfusão no pé. Menos evidente mas no mesmo sentido, o aumento de perfusão registada com a flexão unipodal mas, observada também no membro contralateral. O presente estudo confirmou o interesse da utilização do TiVi nestes domínios, bem como a adequabilidade deste desenho experimental para procurar conhecer mais profundamente o impacto do movimento do membro inferior sobre a dinâmica funcional do pé.

Palavras-chave: microcirculação do pé, LDF, TiVi, ortostático, agachamento simétrico, flexão unipodal
The lower limbs and in particular, their extremities, are fundamental for the biomechanical and vascular stability that under normal conditions harmoniously support, all the body load in quasi-static or dynamic conditions, that is, associated or not to movement (1,2). Structural and functional variations that alter the dynamic function of the foot may predispose it to injury (3,4,5). The implications of the orthostatic variation of body positioning in the microcirculation of the lower limb and foot are known (6,7,8,9), but the influence of movement, especially of the lower limb, on the most peripheral vascular dynamics has been little explored (6,10). One of the main limiting factors is technological, related to the evaluation of peripheral microcirculatory function. In most cases, laser-Doppler flow (LDF) or imaging (LDI) are the most frequent choices. Together with photoplethysmography (PPG), LDF and LDI are currently the technological gold standards in these fields, despite their cost and sensitivity (11,12). The recent introduction of a new optical technology, using polarized non-contact light, offers another potential to explore. This TiVi technology continuously detects the concentration of red blood cell variation, even during movement, and can be applied to more extensive areas (23). In previous studies, its use allowed the identification of some alterations in the foot microcirculation caused by the “step-in-place” activity (13,14).

In the present study, the utility of TiVi was further explored, using LDF as the reference technology. The main objective was to evaluate the sensitivity of this new system to orthostatic and dynamic variations, including the load variation caused by squatting and support in a single foot in a group of previously selected healthy participants.

Materials and Methods

Participants

This pilot study involved five healthy volunteers (26.0 ± 6.5 years old) of both genders (three female and two male), selected after the application of previously defined inclusion / non-inclusion criteria. All participants were normotensive, with no signs of peripheral vascular impairment confirmed by Ankle-Brachial Index values between 1 and 1.4 (1.08 ± 0.03 for male participants and 1.07 ± 0.05 for female participants) (15) and with a Body Mass Index (BMI) of 22.9 ± 1.1 kg/m² (16).

O membro inferior e em especial as suas porções mais distais, são fundamentais para a estabilidade biomecânica e vascular que sustenta harmoniosamente, em condições normais, toda a carga corporal em condições quasi-estáticas ou dinâmicas, associadas ou não ao movimento (1,2). Variações estruturais e funcionais que alterem a função dinâmica do pé, podem predispor a lesão (3,4,5). São conhecidas as consequências que a variação ortostática do posicionamento corporal envolvem na microcirculação do membro inferior e do pé (6,7,8,9), mas, a influência do movimento, em especial do membro inferior sobre a dinâmica vascular periférica tem sido pouco explorada (6,10). Um dos principais fatores limitantes encontra-se nas tecnologias de avaliação da função microcirculatória periférica, quase sempre suportadas na análise por laser-Doppler de fluxo (LDF) ou de imagem (LDI). Juntamente com a fotopletismografia (PPG), LDF e LDI são efetivamente o golden-standard tecnológico nestes domínios, apesar do seu custo muito elevado e da grande sensibilidade de registo (11,12). A recente introdução de uma nova tecnologia ótica, utilizando a espectroscopia de luz polarizada, sem contato, oferece um outro potencial de exploração. Esta tecnologia, deteta continuamente a variação da concentração de glóbulos vermelhos, mesmo durante o movimento podendo ser aplicada em áreas mais extensas (23). Ainda que de forma preliminar, a sua utilização em estudos anteriores permitiu identificar algumas alterações na microcirculação, provocadas pela atividade de marca-passo (13,14).

No presente estudo, procuramos aprofundar o interesse de utilização desta tecnologia, utilizando o LDF como tecnologia de referência. O objetivo principal consiste em avaliar a sensibilidade deste novo sistema face a variações ortostáticas e dinâmicas, incluindo a variação de carga provocada pelo agachamento e pelo apoio num único pé, num grupo de participantes saudáveis, previamente selecionados.
The only restriction imposed was the abstention of consumption of alcoholic and caffeinated beverages during the 24 hours preceding the experiment.

The experimental procedures were previously assessed by the Institutional Ethics Committee, respecting the fundamental principles of good clinical practice, in accordance with the Declaration of Helsinki and subsequent amendments for research on humans (17). All participants were informed in advance about the study, its objectives and components, and expressed their agreement to participate through the respective informed written consent.

**Procedure**

Participants were allowed to acclimate to the laboratory temperature and humidity conditions (21 ± 1°C and 40 to 60%, respectively) for about 20 minutes, resting in dorsal decubitus.

Skin blood perfusion was non-invasively measured in both lower limbs in the dorsal region of the foot, and the anterior area of the leg using Laser Doppler Flowmetry (LDF) as the reference system (Periflux PF5010, Perimed AB, Järfälla, Sweden). The LDF probes were placed between the 3rd and 4th toes in the dorsal region of the foot (Figure 1). Perfusion is expressed in arbitrary units of perfusion (BPU’s). Perfusion was also measured by polarized spectroscopy with the Tissue Viability Imaging system (TiVi 700, WheelsBridge AB, Linköping, Sweden). The TiVi system quantifies the concentration of red blood cells (CRBC - TiVi index), expressed in arbitrary units, in a previously selected region of interest (ROI) on all obtained images (Figure 1).

The experimental design involved the sequential application of three protocols to all participants. A wash-out time of 30 minutes, with the participant maintaining a natural sitting position, was applied between protocols.

Protocol I began with signal recording for 5 minutes in the dorsal decubitus position to obtain a stable register for each individual, and subsequently included:
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Figure 1 - Illustrative example of an image obtained with the TiVi system. In addition to the color scale corresponding to the concentration of red blood cells (not shown), the LDF probes applied between the 3rd and 4th toes are also visible in the dorsal region of both feet.

**Figura 1** - Exemplo ilustrativo de uma imagem obtida com o sistema TiVi onde, para além da escala de cores correspondente à concentração de glóbulos vermelhos (não mostrada), são ainda visíveis as sondas de LDF aplicadas entre o 3º e 4º dedos na região dorsal de ambos os pés.

a) Sitting for 3 minutes - Phase I  
b) Orthostasis for 3 minutes - Phase II  
c) Recovery (10 minutes) in sitting position - Phase III.

Phase III was followed by a washout period, after which Protocol II was applied, specifically:

a) Orthostasis for 3 minutes - Phase I  
b) 1 minute sequence of squatting repetitions (~15) - Phase II  
c) Orthostasis for 3 minutes - Phase III.

Following Phase III, a second washout period was made prior to the application of Protocol III, which included:

a) Orthostasis for 3 minutes - Phase I  
b) Unipodal support with hip and knee bending on the left lower limb - Phase II  
c) Recovery in orthostatic position for another 3 minutes - Phase III  

Figure 1 - Illustrative example of an image obtained with the TiVi system. In addition to the color scale corresponding to the concentration of red blood cells (not shown), the LDF probes applied between the 3rd and 4th toes are also visible in the dorsal region of both feet.

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a) Posição de sentado durante 3 minutos - Fase I  
b) Ortostatismo por 3 minutos - Fase II  
c) Recuperação (10 minutos) na posição de sentado - Fase III, dando lugar a um período de washout, seguido de:

Protocolo II envolvendo:

a) Ortostatismo por 3 minutos - Fase I  
b) Sequência de repetições de agachamento durante 1 minuto (~15) - Fase II  
c) Ortostatismo por 3 minutos - Fase III, dando lugar a novo período de washout, seguido de:

Protocolo III, incluindo:

a) Ortostatismo durante 3 minutos - Fase I  
b) Apoio unipodal com flexão da anca e joelho no membro inferior esquerdo - Fase II  
c) Recuperação na posição ortostática por mais 3 minutos - Fase III
Statistics

Descriptive and comparative statistical tests were applied using the SPSS 21.0 software (IBM, Armonk, NY, USA). The Shapiro-Wilk test confirmed a non-normal distribution of the data. For comparisons between limbs, we used the Mann-Whitney U test for independent samples, and the Wilcoxon test for paired samples for comparisons between phases on each leg. A 95% confidence level (p<0.05) was adopted.

Results and Discussion

The results are shown in Table 1, which includes the recorded values of both lower limbs during the execution of the experimental protocol.

As observed, the perfusion measured with LDF did not detect any significant changes related to the postural modifications introduced by Protocol I, although it detected an increase when changing the position from decubitus to sitting (Phase I) and from sitting to standing (Phase II) decreasing later, as expected, from standing to sitting (Phase III). However, in Protocol II, the introduction of squatting caused a very clear, statistically significant increase in perfusion, which remained higher than in Phase I in both feet, although without statistical difference. In Protocol III, the unipodal support involving the hip to knee flexion in the supporting limb was also revealed in both feet, with a statistically significant increase. Here a fast recovery (Phase III) was noted.

Regarding the red blood cell concentration values detected with the TiVi system, we noted the same type of evolution of this variable with the different protocols, as observed with LDF. The only significant increase in the TiVi index was found from the dorsal decubitus position to sitting (Phase I). The differences in the increase from sitting to standing (Phase II), then decreasing as expected from standing to sitting (Phase III), were not significant.

LDF is a reference method, at the origin of much of the published knowledge on microcirculatory physiology and pathophysiology (18,19). Despite its multiple advantages, including its non-invasive nature, without known risks and extensive application in humans (20), the technique is subject to several criticisms. These criticisms result mainly from technical difficulties.

Estatística

Foram aplicados testes de estatística descritiva e não-paramétrica, utilizando o programa SPSS 21.0 (IBM, EUA). O teste de Shapiro-Wilk confirmou uma distribuição não normal dos dados. Para comparações de perfusão entre membros foi utilizado o teste U de Mann-Whitney para amostras independentes. Para comparações entre fases em cada perna foi utilizado o teste de Wilcoxon para amostras emparelhadas. Adoptamos um nível de confiança de 95% (p<0.05).

Resultados e Discussão

Os resultados são disponibilizados na Tabela 1, que inclui os valores registados de ambos os membros inferiores durante a execução do protocolo experimental.

Conforme se observa, a perfusão medida com o LDF não detetou modificações significativas com as alterações posturais introduzidas pelo Protocolo I, apesar de ter detetado o aumento da posição de decúbito para sentado (Fase I) e de sentado para de pé (Fase II) diminuindo depois, como esperado, de pé para sentado (Fase III). Contudo, no Protocolo II, a introdução do agachamento provocou um aumento muito claro, estatisticamente significativo, da perfusão, que se mantém mais elevada que na Fase I em ambos os pés, embora sem diferença estatística. No Protocolo III, o apoio unipodal com flexão da anca e joelho no membro de apoio também se revela em ambos os pés, com aumento estatisticamente significativo. Neste, a recuperação (Fase III) é mais rápida.

No que diz respeito aos valores de concentração de glóbulos vermelhos detetados com o sistema TiVi, verificámos o mesmo tipo de evolução da variável com os diferentes protocolos, conforme observado com o LDF. O único aumento significativo do índice TiVi, verificou-se na posição de decúbito dorsal para sentado (Fase I). As diferenças do aumento de sentado para de pé (Fase II), diminuindo depois como esperado da posição de pé para sentado (Fase III), não foram estatisticamente significativas.

A LDF é um método de referência, na origem de muito do conhecimento publicado sobre a fisiologia e fisiopatologia microcirculatórias (18,19). Apesar das suas múltiplas vantagens, tratando-se de uma técnica não invasiva, sem riscos conhecidos e extensa aplicação em humanos (20), está sujeita a diversas críticas sobretudo resultantes das dificuldades técnicas.
Table 1 - Mean and standard deviation (m ± sd) of flow-related variables registered during the different phases of these experimental protocols, obtained by LDF and TiVi. The comparative statistics regarding Phase I of each protocol is also shown (* - p<0.05; ns - not significant).

| LDF | Right | m ± sd | 4.8 ± 2.1 | 5.1 ± 1.8 | 6.1 ± 2.4 | 5.7 ± 3.1 | 5.8 ± 2 | 18.1 ± 7.7 | 8.4 ± 5.1 | 4.9 ± 1.4 | 8.9 ± 4.5 | 4.9 ± 0.8 |
|-----|-------|--------|------------|-----------|-----------|-----------|--------|-------------|-----------|-----------|-----------|-----------|
|     | Left  | m ± sd | 4.9 ± 1.6  | 5 ± 1.2   | 6.7 ± 2.1 | 5.3 ± 1.5 | 6.1 ± 1.9 | 19.9 ± 6.4 | 7.1 ± 4.3 | 4.6 ± 1.8 | 9.3 ± 6.6 | 5.8 ± 3.2 |
|     | p     | ns     | ns         | ns        | ns        | ns        | ns      | ns          | ns        | ns        | ns        | ns        |
|     | Δ I-II p | -      | ns         | ns        | -         | ns        | -       | -           | 0.01*     | -         | -         | 0.04*     |
|     | Δ I-III p | -      | -          | -         | ns        | -         | -       | 0.5         | -         | -         | 0.15      |

| TIVi (CRBC) | Right | m ± sd | 180.7 ± 9.1 | 232 ± 7.5 | 235.9 ± 30.5 | 208.7 ± 12.5 | 240.9 ± 28.1 | 252.9 ± 29.1 | 240.9 ± 28.1 | 236.8 ± 21.1 | 257.7 ± 28.7 | 237.7 ± 22.6 |
|-------------|-------|--------|-------------|-----------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
|             | Left  | m ± sd | 186.7 ± 2.4 | 235.3 ± 28.6 | 232.5 ± 18.5 | 233.2 ± 10.5 | 233.9 ± 19.9 | 242.5 ± 18.4 | 233.9 ± 19.9 | 233.5 ± 13.4 | 248.6 ± 22.2 | 238.6 ± 12.2 |
|             | p     | ns     | ns          | ns        | ns          | ns          | ns          | ns          | ns          | ns          | ns          | ns          |
|             | Δ I-II p | -      | ns         | ns        | -           | ns          | -           | ns          | -           | ns          | -           | ns          |
|             | Δ I-III p | -      | -          | -         | ns          | -           | -           | 0.5         | -           | -           | 0.15        | -           |

Tabela 1 – Média e desvio padrão (m ± dp) das variáveis relacionadas com o fluxo registadas nas fases dos protocolos experimentais e obtidas por LDF e TiVi. A comparação estatística com a fase I de cada protocolo é também evidenciada (* - p<0.05; ns – não significativo).
related to understanding and treating its signal, especially in non-static conditions. As this is an optical-based technology, the interaction of light with tissues depends on the wavelength of the laser used and its sensitivity to detect related phenomena (21). The recent introduction of TiVi to quantify the concentration of red blood cells in more superficial areas of the skin seems to offer another assessment potential for measuring during movement (5,22,23). Hence, our exploratory study was designed to identify the dynamics of microcirculatory perfusion alteration as a function of posture. Since the first description of perfusion reduction in the leg from dorsal decubitus to the sitting position nearly 50 years ago (24), more recent studies have allowed a deeper knowledge of the impact of posture changes on the distribution of blood in the lower limb (6,7,8,9). The normal tension response to orthostasis and the adaptation for long periods to the standing position ensures the mobilization of considerable amounts of blood to the distal regions of the lower limbs, with decreased venous return and activation of pressure receptors. This response reveals a wide and complex interaction between different sensors and central and peripheral effectors, still far from being fully identified and understood (25). Even in normal individuals, this microcirculatory adaptation is made at various levels and at different depths in the complex microcirculatory structure of the lower limb. However, we should bear in mind (i) that the detection systems we used do not measure flow, i.e., the volume of blood present in a given arterial segment per unit time (11,18,19,20), (ii) nor discriminate the component of the circuit (venous or arterial) (26), and (iii) do not measure at the same depth along the skin (21). Thus, our optical systems provide both venous and arterial flow-related variables, obtained at different depths where LDF seems to show the deepest penetration capacity (27,28).

The modest flow-related changes observed in Protocol I from recovery of the dorsal decubitus were expected and observed in both feet. However, the most significant variations between decubitus and the sitting position were only detected by the TiVi system with its more superficial measurement capacity. The LDF detected variations in the same direction, but without relevant expression. The bilaterally symmetrical squatting exercise induced intense perfusion changes in the lower limb (Protocol II) by increasing the muscle strength (29-31). Both systems recorded these changes in both feet, but only LDF recorded significant differences in perfusion. This difference may result from the

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mobilization of blood to deeper organs, accessible to LDF but not to the more superficially register of the TiVi (18-20,32,33). By transferring the body weight load to one of the lower limbs, as in Protocol III, keeping the movement dynamics in a temporary unipodal support (simulating in part what occurs during walking or in the activity of climbing or descending steps), we continued to observe significant increases in perfusion in Phase II when compared to Phase I although less intense than those observed in the previous protocol. It is important to note that although this load transfer took place in a single limb, a significant increase in perfusion was observed in both feet. This applied exercise seems to cause neural adaptations in muscle activation evoking a faster and more effective motor response (34-36). The contralateral impact recorded after intervention on the other limb is part of the microcirculatory adaptation mechanisms recently described in man as in the mouse (25). The brief stimulation of one limb promotes the cardio-vascular integration that affects both limbs, demonstrating the existence of multiple neuro-regulatory detectors and effectors, central and peripheral, which restore the local microcirculatory homeostasis when modified, as in this case, by displacing the body load to a single limb (25). This observation may be relevant when defining rehabilitation strategies in the clinical setting and should be further investigated.

**Conclusion**

This exploratory study confirms the value of the TiVi system as a tool to evaluate microcirculation. It allows the evaluation of the impact of movement on peripheral microcirculation under controlled conditions, where flow-related variations affect different depth planes. This study also confirms the existence of a perfusion regulation reflex associated with muscular activity affecting both lower limbs, which should be especially relevant for studies involving orthopedic pathology patients.

inferior (Protocolo II) por aumento da força muscular implicada neste exercício (29-31). Ambos os sistemas registaram aquela alteração em ambos os pés, mas apenas o LDF registou diferenças significativas da perfusão. Tal diferença pode resultar da mobilização do sangue para órgãos mais profundos, acessível ao LDF, mas não a outros sistemas que registam mais superficialmente (18-20,32,33). Deslocando a carga para um dos membros inferiores, como se fez no terceiro protocolo, mantendo a dinâmica do movimento num apoio unipodal temporário, (simulando em parte o que acontece durante a marcha ou na atividade de subir ou descer degraus), continuámos a observar aumentos significativos da perfusão na Fase II quando comparada com a Fase I, embora menos intensos que os observados no protocolo anterior. Importa realçar, que apesar deste apoio ter tido lugar num único membro, o aumento significativo de perfusão foi observado em ambos os pés. Este exercício aplicado parece provocar adaptações neurais na ativação muscular evocando uma resposta motora mais rápida e eficaz (34-36). O impacto contralateral registado após intervenção no outro membro, faz parte dos mecanismos de adaptação microcirculatórios recentemente descritos no homem e no ratinho (25). A estimulação mesmo que breve de um dos membros, promove a integração cardio-vascular que afeta ambos os membros, demonstrando a existência de múltiplos detetores e efectores neuro-regulatórios, centrais e periféricos, que restabelecem a homeostase microcirculatória local quando modificada, como neste caso, pela deslocação da carga corporal para um único membro (25). Esta observação pode ser relevante na clínica, na definição de estratégias de reabilitação, pelo que este conhecimento merece ser aprofundado.

**Conclusão**

O presente estudo, apesar de exploratório, confirma o interesse da utilização do TiVi como tecnologia de medição complementar. Permitiu a avaliação do impacto do movimento sobre a microcirculação periférica em condições controladas, em que as alterações de fluxo se processam em diferentes planos em profundidade. Este estudo confirma ainda a existência de um reflexo de regulação da perfusão associado à actividade musculars, afectando ambos os membros inferiores. Este aspecto pode ser especialmente relevante para os estudos focados em pacientes com patologia ortopédica.
Authors’ contributions

LMR, SN, MF and HS, conception and experimental design; SN, MF, and HS, data acquisition and processing; SN and LMR, manuscript organization and final version approval.

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Conflict of interest

The editor involved in the authorship of this manuscript had no participation in the review or decision process. All authors have stated that there are no financial and/or personal relationships that could represent a potential conflict of interest.

Contribuições dos autores

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Conflito de interesses

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