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Femoral artery blood flow and microcirculatory perfusion during acute, low-level functional electrical stimulation in spinal cord injury

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Disclosures

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research has previously been presented at IFESS conference 2017.
Abstract

Objective – Functional electrical stimulation (FES) may help to reduce the risk of developing macro- and microvascular complications in people with SCI. Low-intensity FES has significant clinical potential since this can be applied continuously throughout the day. This study examines the acute effects of low intensity FES using wearable clothing garment on vascular blood flow and oxygen consumption in people with SCI.

Design – Cross-sectional observation study

Methods – Eight participants with a motor complete SCI received 4x3 minutes of unilateral FES to the gluteal and hamstring muscles. Skin and deep femoral artery blood flow and oxygen consumption were measured at baseline and during each bout of stimulation.

Results – Femoral artery blood flow increased by 18.1% with the application of FES ($P=0.02$). Moreover, femoral artery blood flow increased further during each subsequent block of FES ($P=0.004$). Skin perfusion did not change during an individual block of stimulation ($P=0.66$). Skin perfusion progressively increased with each subsequent bout ($P<0.001$). There was no change in femoral or skin perfusion across time in the non-stimulated leg (all $P>0.05$).

Conclusion – Low-intensity FES acutely increased blood flow during stimulation, with a progressive increase across subsequent FES bouts. These observations suggest continuous, low-intensity FES may represent a practical and effective strategy to improve perfusion and reduce the risk of vascular complications.
Key words: Spinal cord injury, functional electrical-stimulation, blood flow, oxygen consumption

Abbreviations: FES (Functional electrical stimulation), SCI (spinal cord injury), DFA (deep femoral artery), NO (nitric oxide)
Introduction

A spinal cord injury (SCI) leads to significant changes in sub-lesional vascular structure and function. Most characteristic changes involve a decrease in conduit artery diameter\(^1\), increased vascular resistance\(^2\), increased arterial stiffness\(^3\) reduced capillarization\(^4\) and impaired cutaneous microcirculation\(^5,6\) in the paralyzed, inactive limbs. Collectively, such vascular changes are associated with endothelial dysfunction and the development of cardiovascular disease, which is a primary cause of death in persons with a SCI\(^7\). Besides the increased risk of cardiovascular disease, below lesion microvascular endothelial dysfunction manifested as impaired cutaneous blood flow also has significant implications for persons with SCI. The incidence and progression of skin breakdown lesions and pressure ulcers in persons with SCI have been attributed to factors that are associated with a reduction in cutaneous microcirculation\(^8\). Interventions that help reverse macro- and microvascular endothelial dysfunction below, and even above, the lesion are therefore of great clinical significance for persons with SCI.

Studies show that elevations in blood flow and shear stress are required for improvement in vascular function and an increase in artery diameter\(^9,10\). Using electrically stimulated leg exercise in individuals with SCI, Thijssen et al. showed evidence of arterial remodeling in areas subject to electrically stimulated muscular contractions, while vascular adaptations were not apparent in the passive, non-stimulated areas of the leg\(^11\). In addition to conduit remodeling, studies have shown that functional electrical stimulation (FES) results in increased muscle mass\(^12\), higher muscular oxidative capacity\(^13\), enhanced capillary supply\(^4\) and improved blood flow\(^2\). This highlights the potency of FES to mediate beneficial adaptations.
Commonly used methods of FES require specialist facilities and trained staff, making regular application difficult, expensive and impractical. A potential alternative is the use of wearable clothing garments with embedded surface electrodes that automatically stimulate muscles when the garment is applied. This also allows for the adoption of low-intensity FES that can be applied for prolonged periods (i.e. during awake hours). Using this approach, an acute bout of FES to the gluteal and hamstring muscles has shown to reduce pressure over the ischial tuberosity\textsuperscript{14} and increase transcutaneous oxygen levels\textsuperscript{15}. To date, no study has directly examined the acute impact of FES using a wearable clothing garment on both micro- and macro-vascular perfusion in people with SCI.

The purpose of this study, therefore, was to examine the acute effects of low-intensity FES (involving the gluteal and hamstring muscles) on deep femoral artery blood flow (i.e. supplying the active muscles) and skin microcirculatory perfusion (i.e. covering the active areas). It was hypothesized that an increase in conduit artery and skin blood flow would occur with muscle stimulation, whilst also having a cumulative effect leading to a gradual increase in baseline perfusion with repeated application of FES.

**Materials and Methods**

**Participants.** Eight male individuals with ASIA A or B classified SCI participated in this study. All participants were outpatients and frequently visited Reade rehabilitation center for checkups with their physician and to participate in sporting activities. All injuries were traumatic in origin and existed for at least 1 year prior to undergoing the study. None of the participants had any known cardiovascular diseases or took any medication known to interfere with the cardiovascular system. Exclusion criteria included individuals with flaccid
paralysis (i.e. inability to activate the muscles through nerve stimulation), a previous history of autonomic dysreflexia during FES (i.e. for safety purposes) and intolerance or contraindication for the use of FES. The local institutional medical ethical board of Reade Rehabilitation center approved the study and all participants provided written informed consent after receiving and understanding full details of the research study. This study is reported in accordance with the STROBE guidelines and conforms to all items on the checklist accordingly (see supplementary checklist).

**Electrical stimulation.** FES was applied using a specially developed garment with embedded surface electrodes (Axiobionics, Ann Arbor, MI, USA), connected to a portable battery-operated stimulator (Neuropro, Berkelbikes, Nijmegen, The Netherlands). All wires and leads were embedded within the seam of the garment to prevent them becoming entangled with the patient. The FES garment was made from elastic lycra and secured to the body using foldable Velcro straps (Fig 1). One surface electrode was positioned at the upper (proximal) part of the gluteal muscle and a second about halfway down the hamstring area, preventing the participants from lying directly on the electrodes with their buttocks. Ultrasound gel was placed in small Velcro pouches to be used as a conductor between the electrodes and the participants’ skin. FES was applied to the right leg only at a standard constant voltage of 150V using 50Hz biphasic impulse frequency to induce a visible tetanic contraction. The amplitude needed to induce a strong muscle contraction depends on muscle denervation and the amount of muscle nerve fibers that can be recruited and activated. Due to the variability between individuals, the current amplitude was subjectively determined by the researcher and individualized for each participant with increments of 5 to 10mA to a level that did not cause discomfort or excessive movement. To minimize muscle fatigue and ensure
continuous muscle contractions, a 1:4 duty cycle, consisting of 1-second stimulation followed by 4 seconds without stimulation for a period of 3 minutes was used\textsuperscript{14}.

**Protocol and testing procedure.** Participants attended the laboratory at Reade rehabilitation center once to undergo testing. Due to sympathetic nervous system activation and the effects on hemodynamics and blood pressure, all participants were asked to refrain from alcohol and caffeine consumption 24 hours prior to testing. On arrival, the protocol and testing procedures were explained in full to each participant. Participants were transferred from their wheelchair to a bed and positioned comfortably in the supine position. Subsequently, the shorts were applied to ensure correct placement of the electrodes. After a 10-minute rest period and before the start of stimulation, baseline measurements were made for oxygen consumption (VO\textsubscript{2}), skin blood flow, and deep femoral artery (DFA) blood flow in the control and intervention leg. After baseline measurements, the protocol included four blocks of stimulation lasting 3 minutes interspersed with 17 minutes of no stimulation (Fig 2). We chose four blocks of stimulation so we could determine the response and potential benefits of repeated exposure to FES (i.e. a pattern that would be applied in practice). Recordings for all measures were collected 1 minute before and 3 minutes throughout stimulation.

Measurements of DFA diameter and blood flow velocity during stimulation were performed in the intervention leg only. Since it was unlikely that FES would alter blood flow in the contra-lateral, non-stimulated leg (i.e. a systemic effect), we did not measure blood flow in the non-stimulated leg.

**Experimental Measures.**

**Femoral artery blood flow.** Velocity and diameter in the right DFA was measured using a 2-dimensional echo Doppler ultrasound. Using a 10-MHz-multi-frequency linear array probe attached to a high resolution ultrasound machine (T3000, Terason, Aloka, UK), optimal
longitudinal B-mode images capturing the lumen/arterial wall interface, along with Doppler velocity measures of the DFA, approximately 2cm from the bifurcation were obtained. Following image acquisition, 1 min baseline imaging was performed in the control and intervention leg. The same examiner performed all measurements and images were recorded for later offline analysis.

Skin microcirculatory perfusion. We used laser Doppler flowmetry (Periflux system 5000, Perimed AB, Järfälla, Stockholm, Sweden) to obtain an index of microcirculatory perfusion. This is a non-invasive technique that enables evaluation of skin microvascular blood flow over a period of time and is sensitive at detecting changes in response to a stimulus. The technique uses a beam of laser light that undergoes a change in wave lengths when it detects moving red blood cells. The specific changes in wavelength are characterized by red blood cell concentration and velocity to give a measurement of skin blood flow expressed as arbitrary perfusion units (PU). After the FES shorts had been applied and the participant was comfortably lying in a supine position, the laser Doppler flowmetry probes were placed at the measurement site. Blood flow was continuously measured at the skin covering the gluteal muscle on the stimulated leg. A small incision was made in the shorts to allow placement of the laser Doppler probe in close proximity to the stimulated muscle and to ensure fixation throughout the protocol.

Oxygen consumption. Oxygen consumption was collected throughout using a facemask connected to an online gas analyser (Oxycon Pro, Jaeger, The Netherlands). Volume and gas concentration calibrations were performed prior to each test. The participants were instructed not to talk during the measurements.

Data Analysis
DFA diameter and blood flow. Post-test analysis of the DFA was performed using custom-designed edge-detection and wall tracking software which is largely independent of researcher bias. Thorough details of the analysis technique have been described elsewhere. Briefly, data collected on the ultrasound machine were stored as a digital avi file. Subsequent software analysis of the data was performed at 30 Hz using an icon-based graphical programming language and toolkit. The initial phase of analysis required selecting an optimal region of interest (ROI) on the B-mode image, which allowed for automated calibration of artery diameter. Within the ROI, a pixel density algorithm automatically identified the angle corrected near and far wall e-lines. Finally a ROI was drawn around the Doppler waveform and automatically detected the peak of the envelope for this waveform. The mean diameter measure was calculated from within the B-mode ROI and synchronized with the velocity measure which was calculated from the Doppler ROI at 30 Hz. The product of this (artery cross-sectional area and Doppler velocity) gives a measure of average blood flow (mL/s). We have shown that analysis using this semi-automated method produces reproducible diameter calculations that are significantly better than manual methods and producing an intra-observer coefficient of variation of 6.7%.

Skin microcirculatory perfusion. Dedicated software (Perisoft for Windows) was used to collect, store and analyze the skin blood flow data. Unwanted artefact in the data due to participant/wire movement was identified and removed from the data prior to analysis. Resting values were calculated by averaging the last 3 minutes of rest before the start of the next stimulation block, whilst perfusion during stimulation was presented as averages every 30-s.
Oxygen consumption. Five-second bins of gas analysis data were exported to Excel. Steady state average values were calculated from the last minute of rest prior to stimulation and during the entire 3 minutes of stimulation.

Statistical Analysis

Statistical analysis was conducted using the Statistical Package for the Social Sciences. All data were expressed as means ± SD and statistical significance was set at $P<0.05$. Linear mixed models were used to examine the impact of FES on femoral artery and skin microcirculatory blood flow (main effect of “stimulation”: baseline vs stimulation), but also whether the stimulation-induced changes differed across the 4 blocks of stimulation (main effect for “blocks”). The repeated covariance type was compound symmetry and stimulation, blocks and stimulation*blocks were specified as fixed effects and as estimated marginal means. The test of fixed effects stimulation*blocks interaction was interpreted. Significant main effects of stimulation, blocks and stimulation*blocks interaction were followed up with a simple main effects analysis and the least significant difference (LSD) approach to multiple comparisons.

Results

Conduit artery. There was a significant main effect of stimulation on DFA blood flow ($P=0.02$). On average, arterial blood flow increased by 18.1% from 4.69 mL/s at first baseline (pre-intervention) to 5.52 mL/s during 3 minutes of FES (Fig 3). There was also a significant main effect for “blocks” ($P=0.004$), indicating that perfusion at each subsequent baseline and perfusion during stimulation was different across repeated blocks. More specifically, blood flow in block 2 ($P=0.02$), 3 ($P=0.01$) and 4 ($P<0.001$) were all significantly higher than during block 1. There was no stimulation*block interaction ($P=0.74$).
To assess changes in arterial blood flow in the control leg we used a paired samples t-test.

Femoral blood flow in the control leg did not change from pre (3.86±1.66 mL/s) to post-stimulation (3.64±1.52 mL/s; \( t_3 = 0.97, P = 0.41 \)).

Skin blood flow. There was no significant main effect for stimulation (Fig 4) (\( P=0.66 \)), indicating that there was no immediate change in perfusion with stimulation when compared to baseline. However, perfusion did increase over time with repeated stimulation resulting in a significant main effect for “blocks” (\( P<0.001 \)). Skin blood flow, expressed as perfusion units (PU) significantly increased from block 1 (12±6 PU) to block 2 (17±9 PU; \( P=0.01 \)) and block 3 (22±13 PU; \( P<0.001 \)) and was ~80% higher during block 4 compared to block 1 (22±13 PU; \( P<0.001 \)). Blocks 3 and 4 were also greater than block 2 (\( P=0.004 \), but plateaued between blocks 3 and 4. There was no stimulation*blocks interaction (\( P=0.99 \)).

Oxygen consumption. Oxygen consumption did not change throughout the stimulation protocol. There was no significant main effect for stimulation (\( P=0.98 \)), the number of stimulation blocks (\( P=0.94 \)) or stimulation* block interaction (\( P=0.87 \)).

Discussion

The main finding of this study was that unilateral FES acutely increased femoral blood flow in the stimulated leg, most likely a direct result of the increased oxygen demand of the activated gluteal muscles. Skin microcirculatory perfusion also increased from pre-intervention baseline, although the response was more gradual and was not evident during the 3-minute stimulation blocks. Additionally, resting femoral artery blood flow and skin perfusion both progressively increased with repeated bouts of stimulation. Collectively, these
results indicate that low-intensity FES was effective at inducing hemodynamic changes in the superficial and deep layers of the gluteal region. Since frequent increases in blood flow represent a key stimulus for improvement in micro- and macrovascular function and structure\textsuperscript{10}, these observations warrant further research to examine the potential effects of repeated exposure to low-intensity FES on the vasculature in individuals with SCI.

**Blood flow in Stimulated Leg**

This study is the first to examine conduit artery blood flow and skin microcirculatory perfusion in SCI following acute application of FES using a wearable clothing garment. As anticipated, the results show an immediate increase in deep femoral blood flow, even when performed using our low-intensity FES protocol. These findings are consistent with previous data from studies in able bodied\textsuperscript{18} and individuals with SCI\textsuperscript{19}. These previous studies observed a 95% increase in blood flow in the femoral artery during FES. Although we observed a modest increase of 20%, this difference between studies is most likely attributable to distinct stimulation parameters. Whilst in the current study, only two muscle groups were stimulated using a stimulation level that allowed for muscle contractions without overt limb movement (m=75mA), previous work used whole leg muscle stimulation inducing significant muscle movement and therefore marked oxygen demand of the activated muscles. The co-contractions used in the aforementioned studies are also likely to further increase oxygen demand and contribute to greater arterial inflow and blood distribution throughout the entire limb. Nonetheless, it must be emphasized that the large muscle stimulation with marked movement can only be applied for ~20 minutes. Muscle fatigue and energy source depletion prevents longer duration stimulation, whereas low-intensity FES can be applied throughout the day and night and on a day-to-day basis. Although our protocol only increased blood flow by ~20%, the ability for prolonged exposure to low-intensity FES in individuals with SCI
make the FES-protocol applied in the present study a physiologically significant and potentially clinically relevant stimulus.

An important question relates to the mechanisms responsible for the increase in perfusion. Since the current study found no changes in DFA blood flow in the non-stimulated leg, the possibility of systemic stimuli affecting perfusion (e.g. blood pressure) can be excluded. During muscular contractions, a number of mechanisms are known to regulate arterial blood flow supplying the active muscles. Firstly, an increase in cell metabolism initiates the localized release of vasodilator metabolites such as nitric oxide (NO), prostacyclin, ATP, adenosine and potassium from contracting skeletal muscle and the vascular endothelium\textsuperscript{20, 21}. The release of such compounds initiates vascular smooth muscle relaxation, vasodilation of the artery and a subsequent increase in blood flow to the stimulated region. During exercise, skeletal muscle blood flow increases in proportion with metabolic activity to meet the oxygen demands of the contracting muscle\textsuperscript{22}. Considering the direct relationship between skeletal muscle blood flow and metabolic load, it seems sensible to assume that the small, albeit significant, increase in arterial blood flow is due to the low-intensity stimulation protocol we used.

Another physiological impact of low-intensity FES must be considered. The dynamic and mechanical effect of muscle contractions and relaxations, or the ‘muscle pump’ mechanism, importantly influences blood flow in the vasculature. During muscle contraction, a decrease in venous pressure occurs as venous blood empties from peripheral areas (i.e. the legs) and is propelled to the central circulation\textsuperscript{23, 24}. The emptying of venous segments leads to an increase in arteriovenous pressure gradient facilitating an increase in arterial inflow as the muscle relaxes\textsuperscript{25, 24}. Although this study did not differentiate changes in blood flow during the
contraction and relaxation phases of muscle stimulation, the increase in arterial blood flow may, at least partially, be explained through increased muscle pump activity and increases in the arteriovenous pressure gradient.

Microcirculatory Perfusion
Changes in skin microcirculation occur as a reflex thermoregulatory control mechanism during whole body and/or localized changes in temperature\textsuperscript{26}. In the current study, we observed little change in skin perfusion during an individual block of stimulation. However, the combined effect of consecutive and repeated exposure to stimulation did result in a successive rise in skin perfusion over the duration of the protocol. Considering there was no change in whole body VO\textsubscript{2}, it is unlikely that an increase in core body temperature could explain the progressive rise in skin perfusion. A more likely explanation relates to localized heat production and a subsequent gradual warming of the skin covering the activated muscles. This would result in a sustained rise in skin blood flow during localized heating which, is mediated through the release of NO from the vascular endothelium\textsuperscript{27}. Regardless of any change in skin temperature, previous work confirms a NO mediated increase in skin perfusion in response to FES\textsuperscript{28}. Petrofsky and colleagues observed an increase in skin blood flow during FES that was prevented with the infusion of L-NAME, a NO inhibitor. Although the current study nor Petrofsky \textit{et al.} controlled for potential changes in skin temperature, its contribution to the gradual rise in skin perfusion should not be excluded. Future research should consider the exact mechanisms involved in the increase in skin perfusion during FES.

Oxygen Consumption
There was no change in VO\textsubscript{2} during the stimulation protocol, which is in contrast to other studies using FES whilst sitting or lying\textsuperscript{18,29}. In the current study, only two muscle groups
were stimulated using low level FES for 3 minutes. Given the increase in blood flow, it seems logical that energy expenditure in these muscles increased. However, energy expenditure has previously been shown to increase in a dose response relationship with stimulation intensity and the number of muscles stimulated. The small dose of stimulation adopted in the current protocol may be insufficient to detect a significant increase in whole body VO$_2$. Indeed, previous work that reported higher oxygen consumption upon FES adopted higher stimulation (100 mA and 93 mA), but also stimulated a larger muscle mass$^{18}$. $^{29}$ These previous studies confirm that FES has the potential to increase VO$_2$ and energy expenditure which, is indirectly supported by our observation of increased perfusion, and therefore oxygen delivery to the large muscle mass in the legs and gluteal region. One should also consider that changes in oxygen consumption in the current study (involving unilateral FES) may increase exponentially more when FES is applied in a clinical situation using bilateral stimulation.

**Study Limitations**

The small sample size we used may overestimate the true effect of stimulation on vascular perfusion. That said, our data clearly show a distinctive increase in perfusion with FES and we are therefore confident that the results of this study are representative of the wider SCI population. Secondly, due to equipment failure, we were unable to obtain skin blood flow measures in the contralateral, unstimulated leg. However, the low intensity stimulation protocol we used is unlikely to induce any systemic effects on cutaneous perfusion. This is supported by the absent changes in the deep femoral artery in the contra-lateral, non-stimulated leg. Finally, FES induced autonomic dysreflexia is a potential side effect that may limit its usage in some individuals. Although this was an exclusion criterion in the current study, it has previously been reported to occur at higher current amplitudes (160mA) during FES-assisted hydraulic resistance training exercise.$^{30}$ Blood pressure monitoring is therefore
recommended for novice users.

In conclusion, this study clearly shows an increase in superficial and deep vascular perfusion during low level FES. A ~20% increase in blood flow occurred through the deep femoral artery supplying the gluteal muscles, most likely through local increased oxygen demand and muscle pump activation. The results also show a gradual and consistent increase in skin perfusion over the duration of the protocol. This may represent a potent stimulus when this type of low-intensity FES is applied for several hours. Future work is required whether such physiological changes translate to a clinically relevant effect, especially given its simplicity and ability for home-based, day-to-day use.


between young and older humans. American journal of physiology Heart and circulatory physiology 2009;296(1):H57-64.


Figure Legends

Figure 1: Example of electrical stimulation shorts and how they are worn

Figure 2: Schematic of stimulation protocol

Figure 3: Deep femoral artery blood flow at baseline and during stimulation using low-intensity ES in the stimulated leg. Data are presented for each block of stimulation. Error bars represent standard deviations. *P<0.05 vs. Block 1 #P<0.05 vs. Baseline

Figure 4: Skin blood flow at baseline and during stimulation using low-intensity ES in the stimulated leg. Data are presented for each block of stimulation. Error bars represent standard deviations. *P<0.05 vs. Block 1 †P<0.05 vs. Block 2
### TABLE 1. Characteristics of SCI individuals

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (yr.)</th>
<th>Level of injury</th>
<th>ASIA score</th>
<th>Time Since Injury (yr.)</th>
<th>Stimulation level</th>
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<tbody>
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<td>T9</td>
<td>A</td>
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<td>C6</td>
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<tr>
<td><strong>Mean</strong></td>
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<td><strong>-</strong></td>
<td><strong>-</strong></td>
<td><strong>14</strong></td>
<td><strong>75mA</strong></td>
</tr>
</tbody>
</table>
Figure 1.
Figure 2
Figure 3

Blood Flow (ml/s)

Figure 3
Figure 4

Blood flow (perfusion units)

- Baseline
- Stimulation

- Stimulation: P=0.66
- Blocks: P<0.001
- Stimulation*Blocks: P=0.99

* Stimulation
†† Blocks