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Non-invasive and *in vivo* assessment of upper and lower limb skeletal muscle oxidative metabolism activity and microvascular responses to glucose ingestion in humans.

Rogério Nogueira Soares\(^1\), Alessandro L Colosio\(^2\), Juan Manuel Murias\(^1\), Silvia Pogliaghi \(^2\).

\(^1\) Faculty of Kinesiology, University of Calgary, 2500 University Dr. NW, Calgary, AB, Canada.

\(^2\) Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona, Via Felice Casorati, 43, 37131, Verona, VR, Italy.

Corresponding author:

Dr Silvia Pogliaghi

Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona, Italy. Address: Via Felice Casorati, 43, 37131 Verona VR

Email: silvia.pogliaghi@univr.it
Abstract

This study investigated changes in muscle oxidative metabolism and microvascular responsiveness induced by glucose ingestion in the upper and lower limb using near-infrared spectroscopy (NIRS). Fourteen individuals (27±1.4 years) underwent five vascular occlusion tests (VOT) (pre, 30, 60, 90 and 120 min after glucose challenge). NIRS-derived oxygen saturation (StO$_2$) was measured on the forearm and leg muscle at each VOT. Muscle oxidative metabolism was determined by the StO$_2$ downslope during cuff inflation (deoxygenation slope); microvascular responsiveness was estimated by the StO$_2$ upslope (reperfusion slope) following cuff deflation. There was a significant increase in arm (p<0.05; 1-β=0.860) and leg (p<0.05; 1-β=1.000) oxidative metabolism activity as represented by the faster deoxygenation slope at 60, 90, and 120 min (0.08±0.03, 0.08±0.03, 0.08±0.02 %·s$^{-1}$, respectively) (leg) and at 90 min (0.16±0.08 %·s$^{-1}$) (arm) observed after glucose ingestion when compared to their respective pre values (leg= 0.06±0.02; arm= 0.11±0.04 %·s$^{-1}$). There was a significant increase in arm (p<0.05; 1-β=0.880) and leg (p<0.05; 1-β=0.983) reperfusion slope at 60 (arm= 3.63 ± 2.1 %·s$^{-1}$; leg= 1.56±0.6 %·s$^{-1}$), 90 (arm= 3.91±2.1 %·s$^{-1}$; leg= 1.60±0.6 %·s$^{-1}$) and 120 min (arm= 3.91±1.6 %·s$^{-1}$; leg= 1.54±0.6 %·s$^{-1}$) when compared to their pre glucose ingestion values (arm= 2.79±1.7 %·s$^{-1}$; leg= 1.26±0.5%·s$^{-1}$). Our findings showed that NIRS-VOT technique is capable of detecting postprandial changes in muscle oxidative metabolism activity and microvascular reactivity in the upper and lower limb.

Bullet point:

NIRS-VOT is a promising non-invasive clinical approach that may help in the early, limb-specific detection of impairments in glucose oxidation and microvascular function.
**Keywords:** NIRS, glucose, microvasculature, oxidative metabolism, skeletal muscle, oxygen consumption.
1. Introduction

The impairment of skeletal muscle glucose metabolism increases the exposure to glucose in the vasculature following a meal and is associated with vascular dysfunction and increased risk for cardiovascular disease (CVD) (Suzuki et al. 2012). Additionally, the vascular dysfunction associated with CVD reduces the vasodilatory response to glucose ingestion, thereby impairing glucose uptake, metabolism and oxidation by the skeletal muscle in a self-amplifying vicious cycle (Muniyappa et al. 2007). Therefore, being able to identify altered responses to glucose ingestion at the level of the skeletal muscle oxidative metabolism activity and microvascular may offer a valuable tool for the early detection and monitoring of cardiovascular risk and disease.

Glucose ingestion is associated with a phenomenon called diet-induced thermogenesis (Westerterp 2004), which is a metabolic response to glucose absorption (proteins and lipids also induce thermogenesis), metabolism, and oxidation, with skeletal muscles being the main site of glucose oxidation during the postprandial state (DeFronzo and Tripathy 2009; Westerterp 2004). However, most of the studies assessing changes in oxidative metabolism induced by glucose ingestion are either invasive (Kelley and Mandarino 1990; Kelley et al. 1999; Kelley et al. 2002; Simoneau and Kelley 1997), which exposes the participants to discomfort and risk, or conducted using animal models, that do not properly mimic human physiology or disease (Kelley 2005; Kelley and Mandarino 1990; Simoneau and Kelley 1997; Westerterp 2004).

In this context, previous studies have shown that near-infrared spectroscopy (NIRS), combined with a vascular occlusion test (VOT) (NIRS-VOT) provides a valid and non-invasive technique for the in vivo evaluation/monitoring of the muscle oxidative metabolism activity in fasting conditions and during the postprandial period in different populations (Ryan et al. 2014;
Soares et al. 2017c). It has been shown that high-dose glucose ingestion increases the skeletal muscle oxidative metabolism activity in lean individuals while it results in a slight reduction in the leg muscle response in obese participants (Soares et al. 2017c). Furthermore, glucose intake has been associated with acute changes in vascular responsiveness at the conduit arteries (Suzuki et al. 2012) and microvascular level (Soares et al. 2017a; Soares et al. 2017d). For instance, previous studies using NIRS-VOT have shown an increased reperfusion rate in the microvasculature of the tibialis anterior of both healthy and obese individuals after glucose ingestion. Such increased reperfusion rate was sustained for longer after glucose ingestion in the obese group when compared to lean counterparts (Soares et al. 2017a; Soares et al. 2017d).

The abovementioned studies show the usefulness of the NIRS-VOT technique for non-invasively assessing muscle oxidative metabolism activity and microvascular responses to glucose ingestion. However, some limitations should be considered: i) The lack of measurements of body temperature, systemic oxygen consumption (VO$_2$), and respiratory exchange ratio (RER) on these previous studies did not allow for time-resolved comparisons between whole-body and skeletal muscle oxidative metabolism activity; ii) previous measures were only performed in the leg (i.e., tibialis anterior (TA) muscle). However, skeletal muscles from different districts can differ in fiber type composition, which may in turn affect the metabolic and microvascular responses to glucose ingestion. For example, the flexor digitorum superficialis (FDS) muscle in the forearm shows ~30% lower type I fiber (fibers characterized by high capacity for glucose oxidation) occurrence compared to the TA muscle (Albers et al. 2015; Henriksson-Larsén et al. 1983; Hwang et al. 2013; Kong et al. 1994). Also, lower limb vasculature is commonly exposed to higher hemodynamics forces and is less distensible than the upper limb vasculature, which
could make the upper limb microvasculature more responsive to the vascular effects of glucose ingestion (Newcomer et al. 2004; Proctor and Newcomer 2006).

Thus, this study aimed to explore the influence of glucose ingestion at the systemic level (i.e., whole body) and at the “local” muscle level (i.e., arm and leg) by using the non-invasive NIRS-VOT technique combined with an oral glucose tolerance test (OGTT). We hypothesized that the TA muscle in the leg would show a more prominent increase in oxidative metabolism activity in response to glucose ingestion compared to the intrinsically less oxidative FDS muscle in the forearm. Additionally, we would also expect a more pronounced change in vascular reactivity in the intrinsically more compliant forearm compared to the leg during the postprandial period.

2. Materials and Methods:

2.1 Participants

Fourteen male participants (27 ± 1.4 years of age) who had not been diagnosed with any disease or were not taking any medication that affects the cardiovascular system took part in this study. Participants who were smokers, had a BMI lower than 18.5 or higher than 29.9, or who had systolic blood pressure above 139 mmHg and/or diastolic blood pressure above 89 mmHg were excluded from the study. All participants read and signed an informed consent form, prior to the experiment. The study was conducted according to the principles established in the declaration of Helsinki and was approved by the Ethics Committee of the Department of Neurosciences, Biomedicine and Movement Sciences of the University of Verona.

2.2 Experimental procedure
Upon arrival to the laboratory the participants’ body weight, height, and skinfolds (tibialis anterior, flexor digitorum superficialis, abdominal, triceps, suprailliac, thigh, and chest) measurements were taken and information about their physical exercise habits were obtained. Afterward, the participants laid supine quietly on an examination table for 10 min. After this resting period, pre glucose ingestion measurements were taken: blood pressure (BP), blood glucose concentration, body temperature, VO₂, RER, muscle oxidative metabolism activity and microvascular responsiveness. After the pre measures were completed, the participants ingested 75 g of glucose dissolved in 300 mL of water and remained laying down in the supine position throughout the protocol. Blood glucose concentration, body temperature, VO₂, RER, muscle oxidative metabolism activity and microvascular responsiveness measurements were repeated at 30, 60, 90, and 120 minutes after glucose ingestion. All participants were tested between 8 – 9 am and were asked to fast and avoid exercising for 12 hours prior the intervention.

2.3 Measurements

**Blood Pressure, skinfolds, blood glucose concentration, and body temperature**

Body weight and height were measured using a scale and a stadiometer, respectively. Skinfold thicknesses were measured over the muscles to the nearest 0.1 mm with a skinfold calliper and analyzed as previously described (Fontana et al. 2015). Blood pressure, glucose concentration, and body temperature were obtained during the last 2 minutes prior to each VOT. Blood pressure was assessed by using an appropriately-sized blood pressure cuff and sphygmomanometer according to the guidelines from the American Heart Association. Capillary blood samples (20 μl) were collected from the ear lobe and immediately analysed using an electro-enzymatic technique to assess blood glucose concentration (Biosen C-Line, EKF Diagnostics, Barleben, Barleben).
Germany) as previously described (BELLOTTI et al. 2013). Body temperature was obtained using an infrared tympanic thermometer (ThermoScan®, Kaz Europe Sarl, 1003 Lausane, Switzerland).

2.3.1 Oxygen consumption and RER

Breath-by-breath pulmonary gas exchange and ventilation were continuously measured using a metabolic cart (Quark b², Cosmed, Italy); VO₂ and RER were determined as mean values during the last 2 minutes prior to each blood flow occlusion period as previously described (Fontana et al. 2014).

2.3.2 Near-infrared spectroscopy

StO₂ of the TA and FDS muscle were monitored continuously throughout each VOT test (see later description) with a frequency-domain multidistance NIRS system (Oxiplex TS, ISS, Champaign, Ill., USA), as previously described (De Roia et al. 2012).

2.3.3 Muscle oxidative metabolism and microvascular responsiveness assessment

Skeletal muscle oxidative metabolism and microvascular responsiveness were assessed during a VOT as previously described (Soares et al. 2017a; Soares et al. 2017b). Briefly, the first NIRS probe was placed on the muscle belly of the TA muscles and the second one on the belly of the FDS muscle, secured with an elastic strap. An elastic tensor bandage was loosely wrapped around the site to minimize probe movement and light intrusion. A pneumatic cuff connected to an automatic rapid inflation system (constituted of a digitalized pressure regulator, a switch, and a compressor) was placed below the knee (approximately 5 cm distal to the popliteal fossa) and
another cuff connected to the same system was placed above the elbow (approximately 5 cm above the cubital fossa) to induce the occlusion of blood flow. The participants remained laying down with the NIRS probes and inflation cuffs placed on the leg and arm throughout the experimental protocol. The cuffs were inflated to 300 mmHg for the 5 min occlusion period and NIRS measurements were collected continuously at an output frequency of 2 Hz for the duration of the VOT (2 min of baseline, 5 min of occlusion, and 8 min following cuff release) at pre, 30, 60, 90, and 120 min after glucose ingestion (Figure 1) (Soares et al. 2017d).

An index of the muscle oxygen consumption was derived from the downslope of the oxygen saturation signal (StO₂), over a 180s period immediately following cuff inflation (deoxygenation slope, %sec⁻¹) (Soares et al. 2017c). In the absence of blood flow, the rate of decrease of the StO₂ signal represents oxygen extraction in the area of NIRS interrogation.

Microvascular responsiveness was evaluated as previously described (Soares et al. 2017a). Briefly, the StO₂ reperfusion rate was quantified as the slope of the linear increase of the StO₂ signal over a 10s period immediately following cuff release (reperfusion slope, %sec⁻¹).

2.4 Statistical analysis

Based on a mean and standard deviation value of the most variable of our main outcomes, i.e. StO₂ reperfusion slope (%sec⁻¹) at Pre (1.07 ± 0.3 %sec⁻¹) and at 90 minutes (1.53 ± 0.4 %sec⁻¹) after glucose ingestion found in healthy individuals in a similar study (Soares et al. 2017d) and considering a type I error rate of 5% (2-tailed) the sample size required to obtain a power of 80%, was 11 participants. Data are presented as mean ± standard deviation. All data were tested for normality using D’Agostino & Pearson normality test (D'agostino et al. 1990). A paired student’s t-test was applied for skinfold comparisons between the TA and FDS muscles.
Blood glucose concentration, body temperature, VO\textsubscript{2}, and RER data at the different time windows (pre vs 30, 60 90 and 120 min) were compared by one-way repeated measures ANOVA (Huck and McLean 1975). For deoxygenation and reperfusion slopes between limb and time windows comparisons (pre vs 30, 60 90 and 120 min) a one-way repeated measures ANOVA was performed. Dunnett’s post hoc test with corrections for multiple comparisons was applied when appropriate. A Pearson product-moment correlation coefficient or Spearman rank correlation coefficient were used for correlation analysis between the variables of interest (deoxygenation and reperfusion slopes, VO\textsubscript{2}, RER, body temperature, blood glucose concentration, and skinfolds). A \( p \) value \(<0.05\) was considered as the level of statistical significance. Data analysis was performed using the SPSS 23.

3. Results

The general characteristics of the 14 participants who took part of this study were: age (27 ± 1.4 years), body mass index (23 ± 2), systolic blood pressure (126 ± 12 mmHg), diastolic blood pressure (72 ± 9 mmHg), body fat percentage (13 ± 5.4 %), skinfold on the flexor digitorum superficialis (4.5 ± 2.1 mm), and skinfold on the tibialis anterior (5.7 ± 1.7 mm). Regarding the physical activity level, all of the individuals reported to be engaged in upper and lower limb resistance training for at least 4 sessions/week. There was no significant correlation between the FDS skinfolds and the deoxygenation (\( r = 0.213; p > 0.05 \)) and reperfusion (\( r = 0.024; p > 0.05 \)) slopes in the arm at pre. Also, no significant correlations were found between the TA skinfolds and the deoxygenation (\( r = 0.174; p > 0.05 \)) and reperfusion (\( r = -0.189; p > 0.05 \)) slopes in the leg at same the time point.

Table 1 and Figure 2-A show that glucose concentration peaked at 30 min, gradually returning to baseline within 120 min from glucose ingestion. Body temperature increased
significantly at 30 min compared to pre and remained significantly elevated up to 120 min after the glucose intake (Table 1 and Figure 2-B). VO\textsubscript{2} was significantly greater than pre at 30 min, 60 min, and 90 min and returned to baseline at 120 min after glucose ingestion (Table 1 and Figure 2-C). Table 1 and Figure 2-D show that the RER significantly increased compared to baseline only after 60 min from glucose load, remaining significantly elevated up to 120 min post ingestion.

Table 1 and Figure 3-A show that the muscle oxidative metabolism in the arm was increased significantly at 90 min after glucose ingestion when compared to pre. On the contrary, the muscle oxidative metabolism activity in the leg increased significantly at 60 min compared to pre, and remaining elevated up to 120 min following glucose intake (Table 1 and Figure 3-B). Between limbs comparisons showed that the oxidative metabolism activity of the arm was significant higher at all time points when compared to the leg.

Table 1 and Figure 4 depict the time course of microvascular reactivity (reperfusion slope) during OGTT in arm and leg. The reperfusion slope of the arm and leg increased significantly at 60 after glucose ingestion compared to the pre and remained elevated up to 120 min (Table 1 and Figure 4-A and B). Furthermore, between limbs comparisons showed that the vascular reactivity of the arm was significant larger at all time points when compared to the leg (Table 1).

4. Discussion

This is the first study to investigate the changes in muscle oxidative metabolism and microvascular responsiveness induced by glucose ingestion using the \textit{in vivo} and non-invasive NIRS-VOT technique, in two vascular districts (i.e., arm and leg). The main findings of this
experiment were that: i) NIRS-VOT technique was capable of detecting limb-specific changes in oxidative metabolism activity and microvascular responsiveness induced by glucose ingestion; ii) The TA muscle showed an earlier and more sustained increase in oxidative metabolism after glucose ingestion when compared to the FDS muscle; iii) Whole body oxidative metabolism activity showed an earlier increase in response to glucose ingestion when compared to that observed at the level of the muscles;

The findings of the present study are in agreement with a previous investigation showing an increase in the oxidative metabolism activity in the tibialis anterior using the same NIRS approach in a very similar population (Soares et al. 2017c). Together, these findings reinforce the reproducibility and usefulness of NIRS-VOT technique for assessment of postprandial changes in muscle oxidative metabolism activity. Regarding the difference in the oxidative metabolism responses to glucose ingestion between the arm and the leg muscles found in the current study, it is likely that these findings are justified by differences in the training status and/or the fibre composition of the upper vs lower limbs (Periasamy et al. 2017; Pogliaghi et al. 2006). Regarding the training status, the majority of the individuals in the current study reported to be engaged in upper and lower limb resistance training; however, the leg muscles are likely to be exposed to additional contractile activity (i.e. standing and locomotion), in relation to the activities of daily living), that may foster the local training responses (Pogliaghi et al. 2006; Proctor and Newcomer 2006). In relation to fiber composition, Hwang et al., (Hwang et al. 2013) showed that the FDS muscle is constituted of approximately 60% of slow twitch fibers type (higher mitochondrial content and more oxidative than the fast twitch fibers); on the contrary, the occurrence of the slow fibers type in the more superficial regions (area of NIRS interrogation) of the TA muscle is much larger, varying from 75 to 90% ((Henriksson-Larsén et al. 1983) . Consistent with this
idea, the current study demonstrated that the TA muscle showed an earlier and more sustained significant increase in oxidative metabolism activity in response to glucose ingestion than the FDS muscle as represented by a 30% at 60, 29% at 90, and 26% at 120 min increase in the deoxygenation slope in the leg compared to the 38% increase in the deoxygenation slope in the arm at 90 min after glucose ingestion. Additionally, the fundamental role of the slow twitch fibers in glucose homeostasis is reinforced by Gaster et al., (Gaster et al. 2001). The authors showed that a reduction in slow twitch fibers content is accompanied by a reduction in glucose receptors (GLUT4) expression in these fibers, which is associated with impairments in glucose metabolism and development of type 2 diabetes (Gaster et al. 2001).

Interestingly, even though the FDS muscle showed to be less responsive to glucose ingestion when compared to the TA muscle, they had a greater oxidative metabolism activity than the TA muscle at all time points. It is likely that the differences in the rate of oxygen utilization during occlusion might be related to the higher heat production and lower blood volume previously observed in the forearm when compared to the leg muscles (Cross et al. 2008; Stoner et al. 1991). Sustaining a higher heat production (metabolic activity) with reduced perfusion would lead the forearm muscle to a higher reliance on oxygen extraction (faster deoxygenation) to support the metabolic demand. However, further studies aiming to investigate the differences in oxidative metabolism activity between arm and legs related to body temperature and blood volume using NIRS are justified.

Contrary to our hypothesis, it was found that the time-course of the effects of glucose ingestion on microvascular responsiveness did not differ between the arm and leg with both limbs showing an increase in reperfusion slope of approximately 28% at 60, 37% at 90 and 32% at 120 min after glucose ingestion. We acknowledge that measurements of blood insulin
concentration in the current study would have provided further information on the individual metabolic response to glucose ingestion. While this is a limitation of the study, it is also well known that increased blood glucose is associated with elevation of circulating insulin (Soares et al. 2017a), that plausibly leads to vasodilation, increase in blood flow and reductions in vascular resistance (Hayashi et al. 2013; Steinberg et al. 1994; Vollenweider et al. 1993). In relation to this, a previous study using strain-gauge plethysmography to compare the blood flow response to insulin infusion also showed that the time-resolved flow response to different doses of insulin was similar in arms and legs (Utriainen et al. 1995). Together, these findings indicate that although the metabolic responses to glucose/insulin can differ between skeletal muscles, the microvasculature of arms and legs shows similar responsiveness to glucose ingestion/insulin stimulus.

The present study also showed differences in microvascular responsiveness between muscles in the arm and the leg, with the arm showing a faster reperfusion rate (steeper reperfusion slope of the $\text{StO}_2$ signal) at all time points. The current results are in agreement with previous findings (Soares et al. 2018) showing an approximately 50% faster reperfusion slope in the arm compared to the leg. Also, Newcomer et al. (Newcomer et al. 2004) found smaller increases in vascular conductance and blood flow in response to intra-arterial infusions of endothelium-dependent and -independent vasodilators in the leg when compared to the arm. Additionally, it has been demonstrated that the lower limb arteries are less distensible and showed increased wall stiffness and pre-capillary resistance when compared to the those in the upper limb (Eiken and Kölegård 2004).

Regarding the whole body metabolic response to glucose ingestion, the postprandial increase in body temperature and $\text{VO}_2$ observed at 30 min after glucose ingestion in the present
study is in agreement with previous studies (Welle et al. 1981; Westerterp 2004), and has been shown to be more related to the glucose absorption, metabolism, and storage than to glucose oxidation (Welle et al. 1981; Westerterp 2004). Also, previous investigations have shown that glucose ingestion increased mitochondrial activity, with skeletal muscles being the main site for glucose oxidation (DeFronzo and Tripathy 2009; Kelley and Mandarino 1990; Simoneau and Kelley 1997; Soares et al. 2017c). Thus, it is likely that the significant increase in RER, combined with the significantly steeper deoxygenation slope at 60 min after glucose ingestion (leg) found in the present investigation, represents an initial step of the glucose oxidation by the skeletal muscles.

4.1 Experimental considerations

Although some authors suggest that adipose tissue thickness (ATT) should be considered when assessing signals derived from NIRS, we did not find a significant correlation between limbs (FDS and TA) ATT and either deoxygenation or reperfusion slopes. This suggests that the small ATT covering these muscles in healthy and non-obese individuals may not be an issue of concern when assessing oxidative metabolism activity and microvascular responsiveness on these regions. Additionally, previous studies have shown a good intraday repeatability and reliability of NIRS-derived StO$_2$ slopes for up to 5 consecutive VOT, separated by 20 min of resting intervals (Fontana et al. 2015; Iannetta et al. 2018; McLay et al. 2016). Therefore, the likelihood that repeated manoeuvres of cuff inflation and release would affect changes in the StO$_2$ slopes during the postprandial period was considered very small. Also, the results of the present study are limited to physically active individuals and further studies in sedentary/clinical individuals constituted of larger sample size would help to better characterize the specific limb responses to glucose ingestion.
In conclusion, this investigation showed that the effects of glucose ingestion on oxidative metabolism activity can be observed in upper and lower limb, with the lower limb (TA muscle) having an earlier and more sustained increase in oxidative metabolism activity after glucose ingestion when compared to the upper limb (FDA muscle). Additionally, although a faster reperfusion rate was found in the microvasculature of the forearm at pre, the time-course of changes in microvascular responsiveness to glucose ingestion was similar between limbs.

Finally, the current findings showed that NIRS-VOT technique is a useful in vivo and non-invasive tool for a time-resolved assessment of muscle metabolic responses to glucose ingestion in different limbs. The reliability and ease of use of this technique makes it a promising clinical approach that may help in the early and district-specific detection of impairments in glucose oxidation, allowing to design limb-specific interventions aiming to treat or prevent more overt disturbance in muscle glucose metabolism.

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Conflict of interest

The Authors declare that there is no conflict of interest

References


Iannetta, D., Inglis, E.C., Soares, R.N., McLay, K.M., Pogliaghi, S., and Murias, J.M. 2018. Reliability of microvascular responsiveness measures derived from near-infrared spectroscopy across a variety of
ischemic periods in young and older individuals. Microvascular Research. doi:https://doi.org/10.1016/j.mvr.2018.10.001. PMID: 30292692


### Tables

Table 1- Mean and SD of the main variables of the study for all time points.

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<th>Variables</th>
<th>Pre (Mean ± SD)</th>
<th>30 min (Mean ± SD)</th>
<th>60 min (Mean ± SD)</th>
<th>90 min (Mean ± SD)</th>
<th>120 min (Mean ± SD)</th>
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<td>Glycemia (mmol·L⁻¹)</td>
<td>4.2 ± 0.2</td>
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<td>6.7 ± 1.7*</td>
<td>5.9 ± 1.1*</td>
<td>5.1 ± 1.1</td>
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<td>Body temperature (°C)</td>
<td>36.7 ± 0.2</td>
<td>36.9 ± 0.2*</td>
<td>36.8 ± 0.2*</td>
<td>36.9 ± 0.2*</td>
<td>36.9 ± 0.2*</td>
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<tr>
<td>VO₂ (ml·min⁻¹)</td>
<td>297 ± 36</td>
<td>333 ± 39*</td>
<td>343 ± 43*</td>
<td>334 ± 44*</td>
<td>324 ± 55</td>
<td>0.963</td>
</tr>
<tr>
<td>RER</td>
<td>0.76 ± 0.05</td>
<td>0.75 ± 0.06</td>
<td>0.82 ± 0.05*</td>
<td>0.83 ± 0.05*</td>
<td>0.83 ± 0.07*</td>
<td>0.830</td>
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Deoxygenation slope (%·s⁻¹)

| Arm                           | 0.11 ± 0.04#    | 0.12 ± 0.04#       | 0.14 ± 0.08#       | 0.16 ± 0.08**      | 0.13 ± 0.06#        | 0.860|
| Leg                           | 0.06 ± 0.02     | 0.07 ± 0.02        | 0.08 ± 0.03*       | 0.08 ± 0.03*       | 0.08 ± 0.02*        | 1.000|

Reperfusion slope (%·s⁻¹)

| Arm                           | 2.79 ± 1.7#     | 3.27 ± 1.4#        | 3.63 ± 2.1**       | 3.91 ± 2.1**       | 3.91 ± 1.6**        | 0.880|
| Leg                           | 1.26 ± 0.5      | 1.43 ± 0.6         | 1.56 ± 0.6*        | 1.60 ± 0.6*        | 1.54 ± 0.6*         | 0.993|

Arm: Flexor digitorum superficialis muscle; Leg, tibialis anterior muscle; Deoxygenation slope, the downslope of the oxygen saturation signal (StO₂) as derived by near-infrared spectroscopy immediately following cuff inflation; Reperfusion slope, upslope of the StO₂ signal immediately following cuff release; *different from pre (within group) (p < 0.05) #different from leg at the same time point

1-β power of the repeated measures analysis of variance for each variable of the study;
**Figures captions**

Figure 1 – Schematic representation of the five non-invasive NIRS-VOT-derived assessments of muscle oxidative metabolism activity and microvascular function throughout the experimental protocol. Arm, forearm muscle (flexor digitorum superficialis); leg, lower leg muscle (tibialis anterior); NIRS-VOT, near-infrared spectroscopy combined with vascular occlusion test assessment of muscle oxidative metabolism activity and microvascular responsiveness.

Figure 2 – Individual values (dot plot) and Mean and SD of blood glucose concentration, body temperature, oxygen consumption (VO$_2$), and respiratory exchange ratio (RER) for each time point. RER, mean values of respiratory exchange ratio during the 2 minutes baseline prior to each blood flow occlusion VO$_2$, mean values of oxygen consumption during the 2 minutes baseline prior to each blood flow occlusion. *different from pre (p < 0.05); The full RM-ANOVA results along with the power of the test are reported in Table 1

Figure 3 – Change in deoxygenation slope of the arm (Panel A) and leg (Panel B) induced by hyperglycemia compared to the pre. Deoxygenation slope is significantly higher in the arm at 90 min after glucose ingestion when compared to the pre condition. Deoxygenation slope in the leg is significantly higher at 60, 90 and 120 min after glucose ingestion when compared to the pre condition. * Indicates a significant difference from pre (within group) # Different from leg at the same time point The full RM-ANOVA results along with the power of the test are reported in Table 1

Figure 4 – Change in reperfusion slope of the arm (Panel A) and leg (Panel B) induced by hyperglycemia compared to the pre. Reperfusion slope is significantly higher in the arm compared to the leg at all time points (# indicates a significant difference from leg at the same time point). For both arm and leg the reperfusion slope steepness increased significantly at 60, 90, and 120 min after glucose intake, when compared to the pre condition. * Indicates a significant difference from pre (within group). # Different from leg at the same time point The full RM-ANOVA results along with the power of the test are reported in Table 1
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*different from pre (p < 0.05);

The full RM-ANOVA results along with the power of the test are reported in Table 1.

281x190mm (300 x 300 DPI)
Figure 3 – Change in deoxygenation slope of the arm (Panel A) and leg (Panel B) induced by hyperglycemia compared to the pre. Deoxygenation slope is significantly higher in the arm at 90 min after glucose ingestion when compared to the pre condition. Deoxygenation slope in the leg is significantly higher at 60, 90 and 120 min after glucose ingestion when compared to the pre condition.

* Indicates a significant difference from pre (within group)
# Different from leg at the same time point

The full RM-ANOVA results along with the power of the test are reported in Table 1
Figure 4 – Change in reperfusion slope of the arm (Panel A) and leg (Panel B) induced by hyperglycemia compared to the pre. Reperfusion slope is significantly higher in the arm compared to the leg at all time points (# indicates a significant difference from leg at the same time point). For both arm and leg the reperfusion slope steepness increased significantly at 60, 90, and 120 min after glucose intake, when compared to the pre condition.

* Indicates a significant difference from pre (within group).

# Different from leg at the same time point

The full RM-ANOVA results along with the power of the test are reported in Table 1