Comparison of leg muscle oxygenation, cardiorespiratory responses, and blood lactate between walking and running at the same speed

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Research Article

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Comparison of leg muscle oxygenation, cardiorespiratory responses, and blood lactate between walking and running at the same speed

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Abstract

**Purpose:** Muscle oxygenation, expressed as muscle oxygen saturation (SmO$_2$), is being increasingly measured in exercise. Whether different gait modes, or movement patterns, of the same load elicit differences in muscle oxygenation is not known. Thus, the aim of the present study was to compare the oxygenation of two leg muscles (vastus lateralis and gastrocnemius medialis), heart rate, respiratory gases, and blood lactate between two gait modes (walking and running) of the same speed and duration.

**Methods:** Ten men walked and run for 30 min each at 7 km/h in random, counterbalanced order. SmO$_2$, heart rate, and respiratory gases were monitored continuously. Blood lactate was measured at rest, at the end of each exercise, and after 15 min of recovery. Statistical analysis was performed through repeated-measure ANOVA. Significance was declared if $p < 0.05$.

**Results:** Heart rate and oxygen consumption were higher in running compared to walking. Respiratory exchange ratio did not differ between gait modes. SmO$_2$ was lower during exercise compared to rest and recovery, in gastrocnemius medialis compared to vastus lateralis, and in running compared to walking. Blood lactate increased during exercise but did not differ between gait modes.

**Conclusion:** Running caused higher deoxygenation in leg muscles (accompanied by higher whole-body oxygen uptake and heart rate) than walking at the same speed (one that was comfortable for both gait modes), thus pointing to a higher internal load despite equal external load. These findings may form the basis for similar comparisons in other healthy or diseased populations.

**Keywords**

Heart rate, lactate, movement pattern, muscle oxygen saturation, oxygen consumption, running, walking
Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>GM</td>
<td>gastrocnemius medialis</td>
</tr>
<tr>
<td>SmO₂</td>
<td>muscle oxygen saturation</td>
</tr>
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<td>VL</td>
<td>vastus lateralis</td>
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Introduction

Technological advances in recent years have provided valuable services to sports sciences by facilitating the measurement and monitoring of numerous physiological and biochemical parameters during exercise, with a strong impact on sport performance and human health. Included in these parameters is muscle oxygenation, expressed as muscle oxygen saturation ($\text{SmO}_2$) and defined as the percentage of total heme molecules (as part of hemoglobin, myoglobin, and cytochrome structure) in muscle tissue that carry bound oxygen at the time of measurement.

$\text{SmO}_2$ is being increasingly measured in exercise as a non-invasive index of muscle metabolism thanks to the development of small wearable devices that use near-infrared spectroscopy to distinguish between oxygenated and deoxygenated heme (Perrey and Ferrari 2018). Specifically, $\text{SmO}_2$ reflects the balance between oxygen supply and consumption in muscle, as well as microvascular reactivity and hemodynamics of muscle tissue (Rodriguez et al. 2018).

The number of studies on muscle oxygenation and exercise has grown steadily in recent decades, as a search in the Scopus and PubMed databases shows. Most of these studies have examined endurance exercise, and many have used running and walking. Rissanen et al. (Rissanen et al. 2012) reported a linear decrease in vastus lateralis (VL) oxygenation from a speed of 5 km/h (walking) to a speed of 15 km/h (running to exhaustion). Hiroyuki et al. (Hiroyuki et al. 2002) found that, compared to rest, oxygenation of VL increased during walking at 4 and 6 km/h, whereas that of gastrocnemius lateralis decreased; then, running at 8 to 16 km/h had a lowering effect on oxygenation in both muscles. Likewise, Lee et al. (Lee et al. 2011) showed that, although VL and gastrocnemius lateralis oxygenation generally
increased during walking at 3.2 to 6.4 km/h, it generally decreased during running at 8 to 14.4 km/h.

Although the aforementioned studies have examined muscle oxygenation in both walking and running, we could find no study testing the two gait modes, or movement patterns, at the same speed so as to examine whether gait mode per se, rather than speed, is responsible for differences in muscle oxygenation. Therefore, the aim of the present study was to compare muscle oxygenation and perfusion of two leg muscles, that is, VL and gastrocnemius medialis (GM), along with heart rate, respiratory gas exchange, and blood lactate concentration, between walking and running at the usual speed of transition from one mode to the other, which, according to the aforementioned (Hiroyuki et al. 2002; Lee et al. 2011) and other studies [e.g., (Neptune and Sasaki 2005)], is around 7 km/h.

Methods

Sample size calculation and participants

Because, as mentioned above, we could find no study comparing muscle oxygenation between walking and running at the same speed, we had no data to perform a power analysis and deduce the necessary sample size for our study. Thus, we intuitively chose to recruit 10 volunteers and, based on their data, perform an interim power analysis using the G*Power software (version 3.1), which would show us if additional recruitment was necessary. We found a partial $\eta^2$ of 0.374 for the main effect of gait mode on SmO$_2$ (see below under Results, Muscle oxygenation). For an $\alpha$ of 0.05, power of 0.8 and average correlation coefficient among repeated measures of 0.4, the software yielded a sample size of 7. For a power of 0.95, the software yielded a sample size of 9. Thus, the study was adequately powered with 10 participants, and no additional recruitment was warranted. These were
young, healthy men, aged 21-26 years, all students at the School of Physical Education and Sport Science, Aristotle University of Thessaloniki, who volunteered to participate after being fully informed and signing a consent form.

Study design
Participants visited the laboratory once, having had a light breakfast up to two hours before. Each participant was initially subjected to anthropometric measurements, followed by two consecutive tests, one involving walking and one involving running. Ergospirometry, SmO₂ monitoring, and blood lactate measurements were performed during testing and are described in detail below.

Anthropometric measurements
Body mass was measured to the nearest 0.1 kg using an electronic balance (Seca, Hamburg, Germany), and height was measured to the nearest 1 cm by a stadiometer fixed to the balance. Percent body fat was estimated by measuring four-terminal bioelectrical impedance through a Bodystat 1500 apparatus (Douglas, United Kingdom). Skinfold thickness of VL and GM were measured with Harpenden metal calipers from British Indicators (West Sussex, United Kingdom) to ensure the validity of the SmO₂ readings, as the devices used can measure tissue oxygenation at a depth of up to 12 mm.

Exercise testing
Exercise testing was performed on a treadmill (H/p/cosmos pulsar 3p 4.0) with integrated ergospirometer (Jaeger Oxycon Pro) and consisted of 1 min of rest, 30 min of either walking or running at a speed of 7 km/h horizontally, 15 min of passive recovery, 1 min of rest, 30 min of, respectively, running or walking at 7 km/h horizontally, and 15 min of passive
recovery. The two gait modes were tested in random and counterbalanced order, and participants executed both without any distress. During testing, oxygen uptake (VO$_2$), non-protein respiratory exchange ratio (RER) and heart rate (HR), through a built-in Polar heart rate monitor, were measured continuously.

Muscle oxygenation monitoring

SmO$_2$ was measured continuously during testing in each volunteer’s dominant leg with two Moxy portable devices (Idiag, Fehraltorf, Switzerland). One was placed on VL, 14 cm above the center of the patella and 3.5 cm outwards of the imaginary line running along the quadriceps. The other was placed on GM, about 11 cm below the knee joint and 3 cm inwards of the imaginary line running along the gastrocnemius. Data were collected wirelessly by use of the Idiag Moxy software every 2 s. The so-called total hemoglobin (tHb) was recorded at the same time. tHb is a dimensionless quantity that reflects changes in muscle blood supply; it is not useful in an absolute sense because it depends on the thickness of the fat layer over the muscle, the relative contribution of myoglobin, and the blood volume relative to muscle volume. Thus, tHb cannot be used to compare different sites; however, it can provide information about changes in blood supply at a single site. In this sense, tHb was monitored with the purpose of adding mechanistic insight into the changes in SmO$_2$.

Blood lactate measurements

Lactate was measured in capillary blood from a fingertip with a Lactate Scout 4 portable analyzer (EKF Diagnostics, Magdeburg, Germany). Measurements were performed immediately before the onset of each exercise test, immediately after its end, and at the end of recovery.
Calculations and statistical analysis

The values obtained from the continuous monitoring of HR, VO$_2$, RER, SmO$_2$, and tHb were averaged every minute. Energy expenditure during exercise was calculated from average VO$_2$ and RER, based on Péronnet and Massicotte (Péronnet and Massicotte 1991). The distribution of all dependent variables was examined by the Shapiro–Wilk test and was found not to differ significantly from normal. Data are presented as the mean ± standard deviation. Significant differences in HR, VO$_2$, RER, tHb in each muscle, and lactate were detected by two-way (gait mode × time) ANOVA. Significant differences with respect to SmO$_2$ were tested by three-way (gait mode × muscle × time) ANOVA. Effect sizes for significant main effects and interactions were determined as partial $\eta^2$ and were classified as small (0.01–0.058), medium (0.059–0.137), or large (> 0.137) according to Cohen. Average RER and energy expenditure during walking and running was compared through paired Student’s $t$ test. To examine the possible presence of an order effect, the aforementioned analyses were repeated with order of exercise test in place of gait mode. The level of statistical significance was set at $\alpha = 0.05$. The SPSS (Chicago, IL, v. 25) was used for all analyses.

Results

Characteristics of participants

Age, body mass, height, body mass index, body fat and thickness of fat layers over the VL and GM muscles are presented in Table 1.

Heart rate

HR (Figure 1) exhibited significant main effects of gait mode and time (both $p < 0.001$; $\eta^2 =$ 0.769 and 0.803, respectively). Values were higher in running compared to walking throughout the exercise and recovery periods and exhibited a rapid increase from rest to the
3rd minute of exercise. Thereafter they remained relatively stable (with a slight incremental trend) until the end of exercise and decreased exponentially during recovery. Walking was performed at 66 ± 4 % of predicted maximal HR (HRmax = 220 – age), while running was performed at 74 ± 6 % of HRmax. HR during running was higher by 12 ± 10 %, as compared to walking.

Respiratory parameters and energy expenditure

VO₂ (Figure 2) showed a significant interaction of gait mode and time, as well as significant main effects of gait mode and time (all $p < 0.001$; $\eta^2 = 0.917$, 0.992, and 0.740, respectively). Values were higher in running compared to walking during exercise, whereas they were identical or very similar between gait modes in rest and recovery. VO₂ exhibited a sharp increase from rest to the 2nd minute of exercise and remained relatively stable until the end of exercise, decreasing sharply within 2 min of recovery. Walking during the period of stable VO₂ (that is from the 3rd to the 30th minute of exercise) was performed at 22.6 ± 2.1 ml/kg/min, while running was performed at 28.6 ± 1.8 ml/kg/min. VO₂ during running was higher by 27 ± 10 %, as compared to walking.

Average RER during the period of stable VO₂ (that is from the 3rd to the 30th minute of exercise) did not differ significantly between gait modes ($p = 0.783$) and was 0.881 ± 0.028. Average energy expenditure during the same period was 6.9 ± 0.7 kcal/kg/h in walking and 8.7 ± 0.5 kcal/kg/h in running ($p < 0.001$). Energy expenditure during running was higher by 27 ± 11 %, as compared to walking.

Muscle oxygenation
SmO$_2$ (Figure 3) displayed significant interactions of gait mode, muscle, and time; gait mode and time; and muscle and time (all $p < 0.001$; $\eta^2 = 0.382$, 0.349, and 0.732, respectively). There were also significant main effects of muscle ($p = 0.004$, $\eta^2 = 0.630$) and time ($p < 0.001$, $\eta^2 = 0.901$). Values in both muscles and in both gait modes were quite similar at rest (averaging 71%). During exercise, SmO$_2$ remained relatively stable in VL but dropped in GM. In addition, SmO$_2$ was lower during running, compared to walking ($p = 0.045$, $\eta^2 = 0.374$), averaging 76% vs 64% in VL and 45% vs 37% in GM. During recovery, SmO$_2$ increased in both muscles and in both gait modes, reaching similar values at 15 min of recovery (averaging 83%, that is, well above baseline).

**Muscle perfusion**

tHb (Figure 4) showed significant interactions of gait mode and time, as well as a significant main effect of time in both VL (both $p < 0.001$, $\eta^2 = 0.540$ and 0.584, respectively) and GM (both $p < 0.001$, $\eta^2 = 0.190$ and 0.215, respectively). Values in both muscles decreased during the first two minutes of exercise and remained relatively constant till the end of exercise. During recovery, tHb increased, with a reversal between gait modes (that is, higher in running during recovery, as opposed to higher in walking during exercise).

**Blood lactate**

A significant main effect of time was found in blood lactate ($p = 0.009$, $\eta^2 = 0.533$, Figure 5). Lactate was higher in exercise (averaging 1.8 mmol/L), compared to rest and recovery (both $p < 0.05$).

**Order effect**
There was no significant order effect on the outcome measures of the study, except for RER and tHb in VL (both $p < 0.001$). RER was higher in the first gait mode (0.90 ± 0.03) compared to the second one (0.86 ± 0.03). tHb was lower in the first gait mode (12.03 ± 0.42) compared to the second one (12.13 ± 0.40).

**Discussion**

The present study examined, for the first time, differences in muscle oxygenation and perfusion of two leg muscles (VL and GM), heart rate, respiratory gas exchange, and blood lactate between two gait modes (walking and running) at the same speed, one that was comfortable for both gait modes. Our main finding is that running caused higher muscle deoxygenation (accompanied by higher whole-body oxygen uptake, energy expenditure, and heart rate) than walking, all with large effect sizes, thus pointing to a higher internal load despite equal external load.

Our finding of a higher energy expenditure of running vs walking at 7 km/h agrees with that of Margaria et al. (Margaria et al. 1963). In fact, the energy expenditure of walking and running at 7 km/h on a horizontal treadmill resulting from their Figure 1 (6.3 and 8.0 kcal/kg/h, respectively) is similar to our data (6.9 and 8.7 kcal/kg/h, see Results above). As for the energy sources used, there seems to have been no difference between gait modes, as judged from the lack of significant differences in RER or blood lactate. The average RER value of 0.881 (Willems et al. 1995) points to an aerobic energy contribution of 62% from carbohydrates and 38% from lipids (Péronnet and Massicotte 1991).

The higher energy cost of running may be explained by the greater vertical displacement of the center of body mass with each stride, which necessitates a more forceful concentric and
eccentric activity of leg muscles. Additionally, biomechanical differences between the two gait types may play a role (Willems et al. 1995), while a higher stimulation of foot mechanoreceptors that provide feedback to cardiovascular control areas in the brain stem (Katayama and Saito 2019) may contribute to the higher HR during running in the present study.

Although it is known that running requires a higher whole-body oxygen consumption than walking at speeds that are comfortable for both gait modes, such information is lacking at the level of muscle. Thus, our finding of a higher deoxygenation of two leg muscles during running is novel and in agreement with the difference in VO$_2$. What is more, this finding locates the difference (at least in part) at the exercising muscles. Combined with the lack of a significant main effect of gait mode on tHb (suggesting no significant difference in blood and, hence, oxygen supply between gait modes), our data corroborate the hypothesis (described above) of higher muscle activity as an explanation for the higher energy cost of running.

While SmO$_2$ was lower during running than walking in both of the muscles that we examined, there was a marked difference in that SmO$_2$ remained relatively stable in VL but dropped in GM during exercise. Since blood supply (as estimated through tHb) to each muscle remained relatively constant throughout exercise (apart from a decrease during the first two minutes), one may conclude that the blood was generally able to match the increased oxygen demand of exercise in VL but not in GM. This difference may be due to the different muscle fiber composition of the two muscles. Johnson et al. (Johnson et al. 1973), in an autopsy study, found that GM had more type I fibers (50.8%) compared to VL (42.3%; mean of surface and deep). Similarly, Edgerton et al. (Edgerton et al. 1975) found that GM and VL contained about 50% and 32% slow twitch fibers, respectively. It is known that type I fibers are more
oxidative, contain more mitochondria, and have a higher blood supply (capillary-to-fiber ratio) than type II fibers (Mougios 2020). Hence, the drop in GM SmO$_2$ during exercise may be explained by a higher use of oxygen to support aerobic energy production in the more abundant type I fibers, as compared to VL.

Interestingly, SmO$_2$ was rapidly restored (within up to 3 min) after exercise in all cases, exceeding the baseline afterwards (Figure 3). This response may be explained by the decrease in oxygen demand and the increase in blood supply (Figure 4) with the cessation of exercise.

Our findings regarding SmO$_2$ (Figure 3) agree with those of Hiroyuki et al. (Hiroyuki et al. 2002): Although they did not compare walking and running at the same speed, they found that, in walking (4 and 6 km/h), oxygenation of VL increased, whereas that of gastrocnemius lateralis decreased; then, in running (8 to 16 km/h) oxygenation decreased in both muscles. Similarly, Rissanen et al. (Rissanen et al. 2012) showed a linear decrease in VL oxygenation from walking (5 km/h) to running to exhaustion (15 km/h).

**Conclusions**

In young, healthy males, running caused higher VL and GM deoxygenation (along with higher whole-body oxygen uptake, energy expenditure, and heart rate) than walking at 7 km/h. Future studies may examine whether these findings hold in women, in other ages, in disease states, and in designs with blood flow restriction, thus forming the foundation for health applications based on different gait modes.

**Declarations**
Funding: This study was supported by funds of the Laboratory of Evaluation of Human Biological Performance, School of Physical Education and Sport Science at Thessaloniki, Aristotle University of Thessaloniki.

Competing Interests: The authors declare no conflict of interest related to this work.

Ethics approval: All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments. The study was approved by the Research Ethics Committee of the School of Physical Education and Sport Science (EC-8/2020).

Consent to participate: Informed consent was obtained from all individual participants included in the study.

Data availability: The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.
References


Table 1. Characteristics of participants (mean ± SD, n = 10)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
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<tr>
<td>Age (years)</td>
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<tr>
<td>Body mass (kg)</td>
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<td>Height (m)</td>
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<td>Body mass index (kg/m²)</td>
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<td>Body fat (%)</td>
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<td>Fat layer over vastus lateralis (mm)</td>
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<tr>
<td>Fat layer over gastrocnemius medialis (mm)</td>
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Figure legends

**Fig. 1** Mean and standard deviation of heart rate, averaged every minute, at rest, exercise and recovery in walking and running

**Fig. 2** Mean and standard deviation of oxygen uptake, averaged every minute, at rest, exercise and recovery in walking and running

**Fig. 3** Mean and standard deviation of muscle oxygen saturation, averaged every minute, at rest, exercise and recovery in walking and running

**Fig. 4** Mean and standard deviation of total hemoglobin, averaged every minute, at rest, exercise and recovery in walking and running

**Fig. 5** Mean and standard deviation of lactate at rest, exercise and recovery in walking and running. *Significantly different from rest and recovery ($p < 0.05$)
Fig 1

The graph illustrates the change in heart rate (b/min) over time (min) during Exercise and Recovery phases for both Walking (○) and Running (●) activities. The graph shows a gradual increase in heart rate during Exercise, followed by a more rapid decrease during Recovery, with Running having a higher heart rate than Walking throughout.
Fig 3

- **Vastus lateralis Walking**
- **Vastus lateralis Running**
- **Gastrocnemius medialis Walking**
- **Gastrocnemius medialis Running**

**Axes**:
- **X-axis**: Time (min)
- **Y-axis**: \(\text{SmO}_2\) (%)

**Legend**:
- Exercise Rest
- Exercise Walking
- Exercise Running
- Recovery Walking
- Recovery Running
Fig 5

- **Rest**
- **Exercise**
- **Recovery**

**Lactate (mmol/L)**

- **Walking**
- **Running**

* indicates significant difference.