Comparison of Oxygen Consumption in Rats during Uphill (Concentric) and Downhill (Eccentric) Treadmill Exercise Tests

Vivien Chavanelle 1, Pascal Sirvent 1, Gaël Ennequin 1, Kévin Caillaud 1, Christophe Montaurier 2,3, Béatrice Morio 2,3, Nathalie Boisseau 1 and Ruddy Richard 2,3,4

1 Clermont Université, Université Blaise Pascal, Laboratoire des Adaptations Métaboliques à l’Exercice en conditions Physiologiques et Pathologiques (AME2P), Aubière Cedex, France; 2 INRA, UMR 1019, CRNH-Auvergne, Clermont-Ferrand, 1 France; 3 UFR Médecine, Clermont-Ferrand, France; 4 Department of Sport Medicine and Functional Explorations, University-Hospital (CHU), Hôpital G. Montpied, Clermont-Ferrand, France

Abstract
The study of the physiological adaptations of skeletal muscle in response to eccentric (ECC) contraction is based on protocols in which exercise intensities are determined relative to the concentric (CON) reference exercise (as percentage of the CON maximal oxygen consumption, or VO2max). In order to use similar exercise protocols in rats, we compared the VO2 values during uphill (CON) and downhill (ECC) running tests. VO2 was measured in 15 Wistar rats during incremental treadmill running exercises with different slopes: level (0%), positive (+15% incline: CON+15%) and negative (-15% incline: ECC-15%); and -30% incline: ECC-30%). Similar VO2 values were obtained in the ECC-30% and CON+15% running conditions at the three target speeds (15, 25 and 35 cm/sec). Conversely, VO2 values were lower (p < 0.05) in the ECC-15% than in the CON+15% condition (CON+15% VO2/ECC-15% VO2 ratios ranging from 1.86 to 2.05 at the three target speeds). Thus, doubling the downhill slope gradient in ECC condition leads to an oxygen consumption level that is not significantly different as in CON condition. These findings can be useful for designing animal research protocols to study the effects of ECC and CON exercise in ageing population or subjects suffering from cardiovascular diseases.

Key words: Exercise, eccentric, running, VO2, rodent.

Introduction
The capacity to transport and use O2 (i.e., the oxygen consumption or VO2) during large muscle dynamic exercise is a key parameter that allows the efficiency of transfer of physiological work to mechanical movement and varies according to speed or slope changes (Pivarnik and Sherman, 1990). VO2 data are fundamental for exercise prescription and, in human protocols, the target exercise load is generally expressed as a percentage of the maximal O2 consumption per minute (VO2max) determined during an incremental concentric (CON) exercise test.

In physiological studies, rodents are widely used for understanding the mechanisms of muscle response to exercise (Armstrong et al., 1983; Lynn et al., 1998). Treadmill running tests in rats are an invaluable tool for investigating experimentally-induced or pathological impairment of exercise performance and during these tests it is possible to modify the exercise intensity by varying the speed, the slope or both. In CON condition, both the treadmill slope and speed affect physiological demand, which is often evaluated through oxygen consumption (Hoydal et al., 2007; Sonne and Galbo, 1980; Wisloff et al., 2001) , therefore, numerous studies using different treadmill slope and speed have been conducted to assess skeletal muscle adaptations to exercise like mitochondrial biogenesis, fatty acid oxidation and contractile proteins phenotype shift (Egan and Zierath, 2013).

However, little is known in rats about the effects of downhill running conditions (i.e. eccentric muscle work, ECC), especially on VO2. To date, only one study deals with VO2 comparisons in downhill and uphill running conditions at different speeds in rats (Armstrong et al., 1983). In this protocol, VO2 were measured using a box mixing chamber and the only slope variation studied did not allow estimating the additional incline required, at a given speed, to obtain similar uphill and downhill (CON versus ECC) VO2 values. However, these metabolic parameters are very useful for understanding the physiological impact of ECC exercise. In humans, it is well known that Uphill VO2/Downhill VO2 ratio is approximately 2 on treadmill (Pivarnik and Sherman, 1990).

Similar references would be interesting in animals allowing researchers to quantify more accurately the metabolic load sustained during downhill running protocols. To our knowledge, only one study (Armstrong et al., 1983) showed that VO2 measured using a box mixing chamber is 1.5 higher in uphill than in downhill runners (treadmill running tests at 30 cm/sec). Using this method (box mixing chambers) for VO2 measurements complicates data collection during incremental treadmill exercise testing with variable speeds and slopes. Indeed, collecting VO2 data for different speeds using these devices would imply full replacement of the air contained in the box after each speed stage, causing a substantial cost of time. Furthermore, the investigators would not have any direct access to the animal. Breath-by-breath method for measurement of VO2 appears as a good way to overcome these issues; however, few publications have reported values in this experimental condition in rats (Musch et al., 1988; Russell et al., 1980).

The cost of running or walking in uphill and downhill condition in humans is well documented, and nowadays most studies in humans use eccentric and concentric training interventions of comparable mechanical power or with different powers that induce similar per-
percentages of VO$_2$ (Dufour et al., 2004; LaStayo et al., 2000; 2003; Perrey et al., 2001).

This work falls within a translational approach of the understanding of the physiological effects of different types of exercises, from the animal model to the humans. In this regard, it is particularly important to compare muscle adaptations in rats following a bout of exercise inducing, just as it is done in humans, either identical mechanical stimulation (same amount of work, speed and slope in both conditions) or equal metabolic stimulation (exercise intensity that induces the same VO$_2$).

Based on the recent scientific literature, it is interesting to note that among the stimuli known to mediate cellular adaptations to physical training (Changes in intracellular Ca$^{2+}$ concentration, alterations in intracellular pH, changes in metabolite concentrations, increased reactive oxygen species production...), some of them are highly dependent on energetic demand, like for example NAD$^+$/NADH and AMP/ATP ratios (White and Schenk, 2012). These factors have been extensively studied in the past years and they have been proven to be significantly involved in training adaptations via activation of signaling pathways like Sirtuins and AMPK (Jessen et al., 2014; Pucci et al., 2013). Therefore, one can postulate that eccentric exercise would induce similar benefits as concentric training only when performed at the same "metabolic intensity". Nevertheless, ECC exercise also activates some other stimuli like reactive oxygen species production with more potency than concentric exercise (Isner-Horobeti et al., 2014) that could compensate for the lack of activation of metabolic sensors. Taken together, these findings highlight the need of methodological studies assessing the energetic cost of concentric and eccentric training performed on traditionally used ergometers like treadmill (with different slope and speed), in order to enable studies comparing physiological adaptations to both training methods with similar metabolic demand.

Therefore, our main objective was to determine which percentage of downhill and uphill slope induces similar VO$_2$ in rats, during a treadmill run.

To this purpose, we calculated the relationship between VO$_2$ and speed or slope variations in rats during an incremental treadmill exercise with various slopes (i.e., CON and ECC conditions) by measuring VO$_2$ using for the first time an innovating breath-by-breath measuring device that allows direct access to the treadmill and the animals, leading to a significant saving of time compared to the box mixing chamber method, and allowing the completion of a continuous run with increasing speed stages.

Based on previous work (Armstrong et al., 1983; Minetti et al., 1994; 2002) we hypothesized that doubling the eccentric slope (and thus doubling the work load, compared to concentric condition) would induce similar metabolic response (i.e. VO$_2$ values).

Methods

Animals
Male Wistar rats (n=18; age: 3 months; mean weight: 447, ± 7 g) were housed 4 per cage and maintained on a 12:12-h light-dark cycle. Food and water were provided ad libitum. Animal facilities and experimental procedures were approved by the Institutional Animal Care (CREMÉA Auvergne) and in accordance with the APS guiding principles for the care and use of vertebrate animals in research and training.

Experimental protocol
The animals were familiarized with running on a motor-driven treadmill whilst wearing our respiratory masks (the specifications of the mask are described below) 10mn/day for ten days. 15 of 18 rats showed good ability to run with the mask at various speeds and inclines (+15%; 0%; -15%; -30%) and were consequently chosen to take part of the protocol. After the habituation period, VO$_2$ was measured during an incremental exercise test performed on a treadmill. After VO$_2$ measurement at rest for 3 min, each rat (the testing order was random) initially ran at a speed of 15 cm/sec and the speed was increased by 5 cm/sec$^{-1}$ every 3 minutes until the rat was unable or unwilling to maintain the pace despite light stimulation with a cane or electricity. Each rat performed a single test per day with an interval of at least 48 hours between tests. In order to ensure VO$_2$ stabilization, the animals had to fully complete the speed stage (i.e. 3 min) for the measure to be validated. Mean VO$_2$ was calculated out of the measurements of the 30 last seconds of the stage. We tested the animals on different slopes: 3 of them (0%, -15% and +15%) were close to the ones used by previous authors (Armstrong et al. 1983) in order to compare our results to previous findings, and the forth one (-30%) was chosen to test our hypothesis.

Metabolic mask and continuous breath-by-breath VO$_2$ measurement
A lightweight mask was fitted over the rat face and was attached behind the ears with a collar (Figure 1). The total mask volume was 75 ml and the total weight of mask and collar was 6,4 g. A small section of polyethylene tubing (4 mm ID) was attached to the top portion of the mask around the rat head to draw ambient air at a constant rate of 2.5-3 L·min$^{-1}$ (depending on the barometric pressure, temperature and rats’ respiratory variables) unidirectionally into the mask just above the rat snout. The mask was leak-free when filled with water. The effluent, respiratory gas was sucked from the mask to a flow-meter with a vacuum pump and analyzed using a modified breath-by-breath respiratory gas analyzer (Medical Graphics CPXUK). The device measured O$_2$ and CO$_2$ concentrations as a percentage out of the incoming continuous flow, therefore only the latter had to be adapted to the rat tidal volume and respiratory frequency, for better sensitivity. The gas analyzer was calibrated with ambient air and a reference gas (16% O$_2$, 2% CO$_2$ + N$_2$) in a room with stable temperature. When the animal breathes, respiratory signal (FO$_2$ and FCO$_2$) can be visualized instantaneously on the computer screen (Figure 2) and the air flow can be manually adjusted for greater sensitivity. The best results were obtained for an air flow of between 2.5 L·min$^{-1}$ and 3 L·min$^{-1}$.)
Figure 1. The effluent gas from the mask was drawn through a flow-meter with a vacuum pump and was analyzed using a breath-by-breath device. The mask fit over the face of the rat and was attached behind the ears with a collar. A small section of polyethylene tubing was attached to the top portion of the mask to draw ambient air into the mask just above the rat’s nose.

Data and statistical analyses

CON VO₂/ECC VO₂ ratios, in which VO₂ was expressed as a relative value (exercise VO₂ – resting VO₂), were calculated at three target speeds (15, 25 and 35 cm/s), that corresponded to about 50, 65 and 75% of the VO₂max. Results are shown as the mean ± SEM. Normality was verified using Shapiro-Wilk’s test. We Log10 transformed data to homogenize variances when it was necessary. Homogeneity of variances was assessed using Levene’s test. Speed and slope effects were analyzed by using a two-way ANOVA. Tukey’s post hoc test was used when the ANOVA yielded a significant F-Ratio. The significance level was set at p < 0.05.

Results

Incremental exercise tests

The number of rats that performed the incremental exercise tests at +15%, 0%, -15% and -30% incline are listed in Table 1. Forty-four incremental exercise tests were thus retained for analysis; however, the number of rats for which we could calculate the relative VO₂ (exercise VO₂ – resting VO₂) in CON (+15%) and ECC (-15%; -30%) conditions at the different target speeds (15, 25 and 35 cm/sec) varied as not all animals could keep running at the highest speeds in all running conditions (see Table 1).

Table 1. Treadmill exercise conditions (slope, and speed) and number of rats in each testing group. In bold, are the target speeds (15, 25 and 35 cm/sec) and the number of rats that managed to perform the exercise at the different speeds.

<table>
<thead>
<tr>
<th>SPEED (cm·sec⁻¹)</th>
<th>SLOPE (%)</th>
<th>15%</th>
<th>0%</th>
<th>-15%</th>
<th>-30%</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>14</td>
<td>5</td>
<td>15</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>11</td>
<td>5</td>
<td>15</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>11</td>
<td>5</td>
<td>15</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>10</td>
<td>5</td>
<td>15</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>9</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>7</td>
<td>4</td>
<td>6</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>-</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>-</td>
<td>3</td>
<td>2</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

VO₂ to work rate relationship

For the four slope conditions (+15%, 0%, -15% and -30% incline), the VO₂ values increased with speed (data not shown). For each target speed, the CON +15% and ECC -30% VO₂ values overlapped.

Maximal Oxygen Consumption (VO₂max)

A VO₂ plateau (VO₂max), defined by the absence of VO₂ rise despite speed increase, was reached in nine tests [VO₂max = 39 ±1 mL·min⁻¹ and 89 ± 2 mL·min⁻¹·kg⁻¹ (mean rat weight: 447 ± 7 g)].
Relative CON and ECC VO$_2$ and CON VO$_2$/ECC VO$_2$ ratios

For the three slope conditions (+15%, -15% and -30%) the relative VO$_2$ and the corresponding CON VO$_2$/ECC VO$_2$ ratios were calculated at 15, 25 and 35 cm·sec$^{-1}$ (Figure 3). No significant difference was found between the CON +15% and ECC -30% mean relative VO$_2$ values at the different speeds (Figure 3a). Conversely, when comparing the CON +15% and ECC -15% conditions, the ECC -15% relative VO$_2$ values were significantly lower at the three target speeds, despite a similar amount of mechanical work. Accordingly, (CON +15% VO$_2$/ ECC -15% VO$_2$) ratio was 1.86 at 15 cm·sec$^{-1}$, 2.05 at 25 cm·sec$^{-1}$ and 1.65 at 35 cm·sec$^{-1}$ (Figure 3b).

Discussion

To our knowledge, this study is the first to show that no significant difference appeared on mean relative VO$_2$ values at the different target speeds when the CON +15% and ECC -30% conditions were compared. Armstrong et al. (1983) only compared oxygen consumption in rats at similar mechanical power (identical CON and ECC load, defined as same slope gradient).

Conversely, the ECC -15% relative VO$_2$ value was significantly lower than the CON +15% VO$_2$ value at the three target speeds, despite a similar amount of work. Accordingly, (CON +15% VO$_2$/ ECC -15% VO$_2$) ratios were found between 1.65 (at 35 cm·sec$^{-1}$) and 2.05 (at 25 cm·sec$^{-1}$).

The lower metabolic cost of ECC exercise is well documented in humans (Piazzesi et al., 1992). At the same mechanical intensity, the ECC muscle contraction generates muscle force with lower metabolic demand (i.e., lower oxygen cost) compared to CON contractions (Dufour et al., 2004; Perrey et al., 2001). This could be due to mechanical and non-ATP-dependent rupture of Actin-Myosin cross-bridges (Piazzesi et al., 1992) and/or to a greater distance covered by each individual Actin-Myosin cross-bridge (Ryschon et al., 1997) during each ECC muscle contractions.

We obtained results comparable to the findings of previous authors (Armstrong et al. 1983) by using a metabolic mask and similar experimental conditions (15% downhill and uphill slopes compared to 17.6% in the previous study). Specifically, when using the same speed as these authors (30 cm/sec), the (CON+15% VO$_2$/ECC-15% VO$_2$) ratio was 1.3 (absolute VO$_2$ values).

Our results indicate that to obtain similar VO$_2$ levels in downhill and uphill running conditions (and thus comparable exercise metabolic intensity), either the speed, as previously described by Armstrong and et al. (1983), or the slope (this study), or both must be increased in downhill (ECC) condition. As the maximal speeds recorded in rats are of about 50-60 m·min$^{-1}$ (Armstrong et al., 1983), increasing the slope may be a good compromise to reach the target VO$_2$.

Conclusion

The CON VO$_2$/ECC VO$_2$ ratio can be useful for designing animal research protocols aiming at comparing the physiological effects of eccentric and concentric exercise at the same metabolic power (defined as the metabolic cost) or at the same mechanical power (defined as the mechanical work or the mechanical force applied on the muscle, thus directly depending on both slope angle and speed, in the case of a treadmill run).

Studies aiming at comparing the effects of concentric and eccentric work are often carried out with reference to concentric (same percentage of VO$_{2\text{max}}$ or heart rate), but the physiological mechanisms that lead to these adaptations require more applied research, especially in animal models. In this study we determined two conditions that may generate comparable metabolic responses in downhill (eccentric) and uphill (concentric) running.
conditions in rats. Our results show that doubling the downhill slope gradient leads to an oxygen consumption level that is not significantly different than in uphill condition. As muscle movement mechanics and metabolism are dependent on speed and incline, these parameters are necessary for the development of future experimental protocols that will focus on the physiological adaptations that occur in the muscle, after an eccentric bout of exercise.

Using this innovative device to compare breath-by-breath VO2 in rats during downhill versus uphill running appears to be of great interest in order to perform more accurate comparisons of the physiological adaptations induced by these exercise training programs. This research area is related to valuable stakes regarding therapeutic potential in chronic disease. Indeed, if beneficial muscular adaptations are obtained with eccentric exercise at lower metabolic intensities, further implications would include the use of physical activity in human populations that cannot bear heavy exercise (elderly people or patients suffering from cardiovascular and respiratory diseases).

References


Key points

- VO2 in rats during treadmill race in eccentric and concentric conditions were measured.
- A novel breath-by-breath device allowing direct access to the animal was used.
- Three different slopes: +15%, -15% and -30% were used.
- VO2 values obtained in the -30% eccentric and the +15% concentric conditions were not significantly different.

AUTHORS BIOGRAPHY

Vivien CHAVANELLE

Employment

Engineer at the AME2P laboratory in Clermont-Ferrand, France.

Degree

MSc

Research interests

Exercise physiology, nutrition and biochemistry.

E-mail: vivien.chavanelle@hotmail.fr

Pascal SIRVENT

Employment

Lecturer, Sports University, UFR STAPS, Clermont-Ferrand and research Laboratory AME2P, Clermont-Ferrand, France.

Degree

PhD

Research interests

Exercise physiology, biochemistry, physical training and nutrition.

Gaël ENNEQUIN

Employment

PhD student at the AME2P laboratory in Clermont-Ferrand, France.

Degree

MSc
**Research interests**
Novel cytokine (Neuregulin), its link to physical exercise and its potential beneficial effects on human metabolism.

**Kévin CAILLAUD**

**Employment**
PhD student at the AME2P laboratory in Clermont-Ferrand, France.

**Degree**
MSc

**Research interests**
Novel cytokine (Neuregulin), and particularly on its implication in human metabolism.

**Christophe MONTAURIER**

**Employment**
Engineer at the Human Nutrition Unit JRU 1019 INRA – Clermont 1 University.

**Degree**
PhD

**Research interests**
Human and animal metabolism

**Béatrice MORIO**

**Employment**
A physiologist at the Human Nutrition Unit JRU 1019 INRA – Clermont 1 University.

**Degree**
PhD

**Research interests**
Prevention and treatment of obesity and its related metabolic disorders, mainly insulin resistance and type 2 diabetes.

**E-mail:**

**Nathalie BOISSEAU**

**Employment**
Prof. and the leader of a research team called “Metabolic Adaptations to Exercise in Physiological and Pathological conditions (AME2P)” belonging to the Human Nutrition Research Centre (CRNH) of Clermont-Ferrand, France.

**Degree**
PhD

**Research interests**
Energy Metabolism, especially with regards to its regulation by exercise and nutrients in healthy individuals or metabolic disorder patients (including women and children).

**Ruddy RICHARD**

**Employment**
Lecturer in the Sport Medicine Division of Gabriel Montpied hospital (Service de Médecine du Sport et des Explorations Fonctionnelles, CHU G Montpied – Clermont Ferrand).

**Degree**
PhD, MD

**Research interests**
Mitochondrial adaptations and oxidative stress following physical activity.

**E-mail:** rrichard@chu-clermontferrand.fr

**Professor Ruddy RICHARD, MD, PhD**
Service de Médecine du Sport et des Explorations Fonctionnelles, CHU G Montpied, 58, rue Montalembert, 63001 Clermont-Ferrand Cedex 1, France