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Non-standard \dot{O}_2 consumption–temperature curves during rest and isometric exercise in human skeletal muscle[☆]

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Abstract

The present work was aimed at measuring intramuscular oxygen consumption (\dot{O}_2) as a function of temperature (T), in human forearm, during rest and aerobic isometric exercise (4% of the maximal voluntary contraction, MVC). Based upon results from in vitro experiments performed on isolated mitochondria of animal species, it was hypothesised that, during isometric exercise, the \dot{O}_2 - T curve should display a maximum for some ‘optimal’ T . Intramuscular T and measurements were performed using a combined deep body temperature/near infrared probe during muscle cooling. At rest, \dot{O}_2 increased non-linearly and monotonically as a function of T ($n=8$). \dot{O}_2 increased ~ 2 times when going from 26 to 36 °C. A $\log(\dot{O}_2)$ - T plot or a $\log(\dot{O}_2)$ - $1/T$ did not linearise the data. During isometric contraction, \dot{O}_2 values at 26.8 \pm 0.6, 28.6 \pm 0.9, 31.9 \pm 0.9 and 35.9 \pm 0.9 °C were 3.04 \pm 1.26, 7.60 \pm 1.64, 4.43 \pm 1.95, and 6.64 \pm 1.37 $\mu\text{mol } 100 \text{ g}^{-1} \text{ min}^{-1}$, respectively ($n=6$). The \dot{O}_2 value at 28.6 °C was significantly higher ($P<0.05$) than that at 26.8 and 31.9 °C. The ‘sudden’ \dot{O}_2 change at 28.6 °C is compatible with the phenomenon observed at the mitochondrial level. © 2002 Elsevier Science Inc. All rights reserved.

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1. Introduction

Muscle function and metabolism are strongly influenced by local temperature (T) changes. As often as not, non-standard rate- T curves are observed. For example, the muscular energy demand or utilisation, the enzymatic activities, etc. may display thermal optima (Cossins and Bowler,

1987; Rall and Woledge, 1990). In this case, classical models, such as the Krogh normal curve, are unsuitable to describe the phenomena. Endurance time (Clarke et al., 1958; Edwards et al., 1972) or maximum force of contraction (Rall and Woledge, 1990) are also T -dependent and present optimal T that are different from 37 °C. Many hypotheses have been formulated to explain these observations, e.g. impaired neuromuscular transmission or T -dependent substrate utilisation, but no definitive conclusions have been drawn. To our knowledge, there is a complete lack of data concerning T -modulation of oxidative metabolism during isometric exercise. Moreover, no theoretical hypotheses exist concerning the shape of the relat-

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ed \dot{O}_2 consumption (\dot{O}_2)- T curve. For these reasons, the present work was aimed at measuring \dot{O}_2 as a function of T , in human skeletal muscle, during rest and aerobic isometric exercise. It was hypothesised that, during isometric exercise, the \dot{O}_2 - T curve should display a maximum for some 'optimal' T , based upon results from previous *in vitro* experiments performed on isolated mitochondria of animal species (Newell and Northcroft, 1967; Davison, 1971).

2. Methods

Eight healthy volunteers participated in this study. A deep-body-temperature (DBT)/near-infrared (NIR) probe, adapted for measurements in water (Binzoni et al., 1999), was placed on the right forearm (hand flexors). The DBT/NIR probe allowed one to measure, simultaneously, intramuscular temperature and NIR spectra in the same region of interest (ROI).

The DBT probe uses a non-invasive method to measure temperature, which employs the zero heat flow principle (Fox and Solman, 1971; Fox et al. 1973; Togawa, 1985). The method is based on the assumption that if the heat flow across a probe attached to the skin surface is zero, there must exist a region of uniform temperature beneath the skin. The zero heat flow condition is achieved by the activation of a heating element integrated into the probe until the temperature of two sensors, one in contact with the skin and the other on the upper side of the probe, are equal, i.e. no heat flow exists across the probe. The temperature of the tissue below the skin, to a depth approximately equal to the square root of the diameter of the probe, is then the same as that of the skin surface sensor.

The DBT/NIR probe was built, combining a standard zero heat flow probe (Deep Body Thermometers Ltd., Little Eversden, Cambridge, UK) to a fibre coupled to a CCD-based near infrared spectrophotometer (Cope et al., 1989). This probe produces a uniform temperature distribution in the ROI (Binzoni et al., 1999) and in this manner reduces the errors in the \dot{O}_2 measurements. The source and detector fibre terminals coming from the spectrophotometer were incorporated into the DBT probe. Light coming from a stabilised tungsten halogen light source (Oriel 77501, Stratford CT, USA) was conveyed to the forearm using one of these two fibre optic bundles, while transmitted

light was collected via the second fibre bundle and focused onto the entry slit of a spectrograph (Spex 270M, Instruments SA Group, Edison, NJ, USA). The interoptode distance was 3 cm. Spectra with a resolution of 0.32 nm/pixel were acquired between 677 and 998 nm on the cooled CCD-based NIR spectrophotometer (Wright Instruments, Enfield, London, UK) and were stored on a PC (Cope et al., 1989). Changes in concentration of oxy- ($[\Delta\text{HbO}_2]$) and deoxyhemoglobin ($[\Delta\text{Hb}]$) were obtained by fitting changes in the attenuation spectra of HbO_2 and Hb, which had been corrected for the wavelength dependence of pathlength (Essenpreiss et al., 1993).

The subject was comfortably seated with the right forearm immersed in a thermostated bath equipped with an isometric ergometer. Intramuscular T , water T and cuff pressure (from a cuff around the upper arm) were monitored continuously on a PC (Real-Time Windows Target, The Mathworks Inc., Natick, MA, USA). \dot{O}_2 was calculated at different T (interval 26–37 °C), by measuring the slope of $[\Delta\text{Hb}]$ changes during 20 s arterial occlusion (see Ferrari et al., 1997; Van Beekvelt et al., 2001, for reviews). \dot{O}_2 measurements were performed both at rest and during isometric contraction (4% of the maximal voluntary contraction, MVC). MVC was determined using a hand dynamometer (Lafayette Instrument Company, Indiana, USA), with the subject installed in the same position as for the experimental protocol. Measurements during isometric contractions were performed on six of the eight subjects, interleaving rest and exercise. The exercise duration was long enough to allow one to follow $[\Delta\text{Hb}]$ and $[\Delta\text{HbO}_2]$ changes during 20 s arterial occlusion (Ferrari et al., 1997; Van Beekvelt et al., 2001) and did not exceed 50 s. The experiment during isometric contractions was repeated a second time on three subjects (see Section 4).

3. Results

The intramuscular T time course for a typical subject is shown in Fig. 1. The estimated maximum T change observed during 20 s was ~ 0.039 °C. This is a negligible value and spectra acquisition performed during this 20 s period can be considered taken at constant T . At rest, \dot{O}_2 increased as a function of T ($n=8$) and the \dot{O}_2

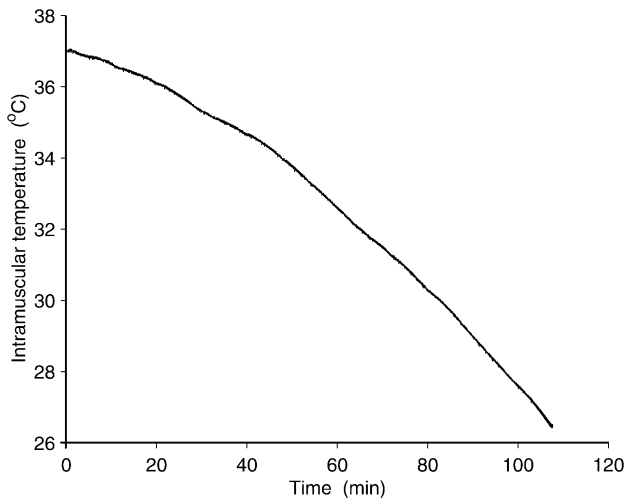


Fig. 1. Intramuscular T time course for a typical subject.

values doubled when going from 26 to 36 °C (Fig. 2). However, a $\log(\dot{O}_2)$ - T plot or a $\log(\dot{O}_2)$ - $1/T$ did not linearise the data (note that in this particular case the temperature scale must be expressed in K).

During isometric contraction, \dot{O}_2 values at 26.8±0.6, 28.6±0.9, 31.9±0.9 and 35.9±0.9 °C were 3.04±1.26, 7.60±1.64, 4.43±1.95, and 6.64±1.37 $\mu\text{mol } 100 \text{ g}^{-1} \text{ min}^{-1}$, respectively ($n=6$, see Fig. 3). ANOVA analysis shows that the \dot{O}_2 value at 28.6 °C was significantly higher ($P<0.05$) than that at 26.8 and 31.9 °C. These mean T -values were chosen to highlight the sudden \dot{O}_2 change from a 'standard' T curve (by 'standard' we mean here \dot{O}_2 T -induced variations without any sudden changes; Cossins and Bowler, 1987).

To better define the shape of the \dot{O}_2 - T curve during isometric contraction, the experiment was repeated again on three subjects, but only with the exercise protocol. In this case, \dot{O}_2 values at as many as possible T were obtained. All three subjects had a similar \dot{O}_2 - T curve. In Fig. 4 the results are shown for one typical subject. Considering the similarity of the results, the fact that the protocol is relatively strenuous for the subjects and the long experimental time (2–3 h) this last procedure was performed only for three subjects.

Total blood volume, defined as $[\Delta\text{HbO}_2] + [\Delta\text{Hb}]$ (Ferrari et al., 1997), was constant over the \dot{O}_2 measurement intervals, (i.e. in 20 s intervals).

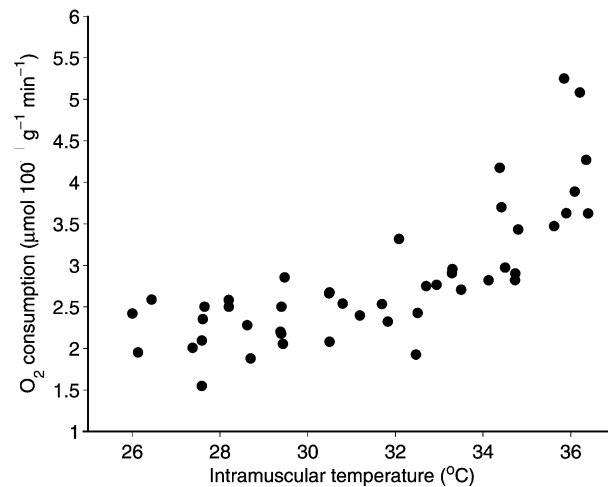


Fig. 2. Oxygen consumption curve as a function of intramuscular temperature of the human forearm during rest ($n=8$). The points represent individual measurements.

4. Discussion

The data reported in Fig. 2 show the results from a group of eight subjects. \dot{O}_2 at 36 °C is apparently twice the \dot{O}_2 at 26 °C. The scatter of the data is probably explained by the possible differences in the optical pathlength among the subjects. In fact, a fixed mean differential pathlength factor (DPF) of five was utilised for the \dot{O}_2 estimation (Duncan et al., 1995). Differences

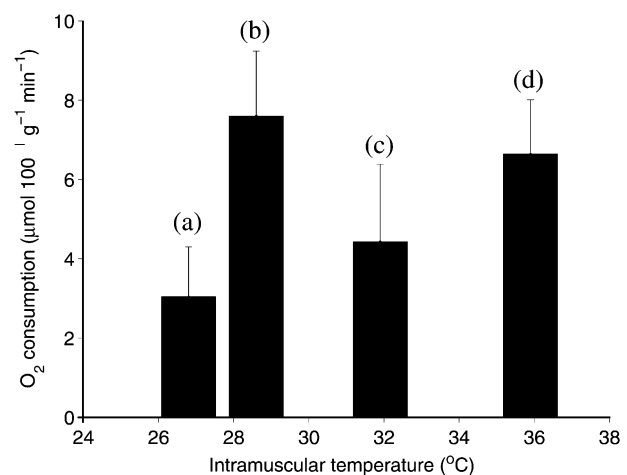


Fig. 3. Oxygen consumption as a function of intramuscular temperature of the human forearm during isometric contraction ($n=6$). (b) is significantly different from (a) and (c) ($P<0.05$). (c) is significantly different from (b) and (d) ($P<0.05$).

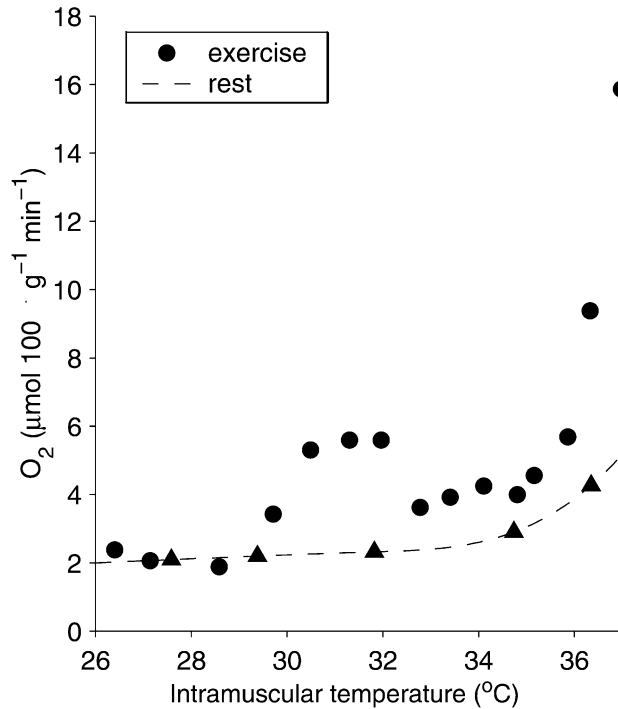


Fig. 4. Oxygen consumption curve as a function of intramuscular (forearm) temperature of a typical subject during rest (dashed line) and isometric contraction (closed circles).

in DPF might also explain the fact that in Fig. 4, \dot{O}_2 is the same for rest and work, at temperatures <29 °C. In this case, as explained in Section 2, the two experiments (rest and isometric exercise) were performed at two different times. This means that the position of the optical fibres might not be exactly the same in the two studies, giving a slightly different DPF and hence slightly displacing one of the two curves (since the DPF is a multiplying factor). This effect is, of course, not present if one compares Figs. 2 and 3, where the experiments were performed simultaneously. In this case, \dot{O}_2 for the isometric exercise is greater than that at rest. It is interesting to notice, that Bottinelli and Reggiani (2000) demonstrated that on isolated human muscle fibres, an isometric contraction increases the cost of force production when increasing temperature. This means that one can hypothesise that at low temperatures, the muscle cell need less \dot{O}_2 for the same mechanical load. The comparison between Figs. 2 and 3 (exercise minus rest), seems to be in agreement with this interpretation. However, further studies will be necessary to confirm this observation.

Changes in DPF as a function of T , for a given subject, were taken into account as proposed in Binzoni et al. (1999). The fact that \dot{O}_2 - T curves at rest cannot be linearised using log or $1/T$ scales means that the observed phenomenon is not simply a thermodynamic 'slow-down' of the metabolic processes. The rate of O_2 unbinding from oxygenated haemoglobin and the Hb- O_2 affinity also depend on temperature. However, this effect should not influence the present measurements. In fact, the measurements were made in a closed system (arterial occlusion). Thus, for every four O_2 molecules metabolised by the muscle one must have one Hb O_2 /Hb molecule disappearing/appearing. The dissolved O_2 is negligible in the present case ($\sim 1\%$ of the total haemoglobin; West, 1990). The rate of O_2 unbinding from oxygenated haemoglobin remains, in any case, very fast compared to muscle O_2 consumption rates (Baumann et al., 1987) and should not be a limiting factor on \dot{O}_2 .

It is noteworthy that the results shown in Figs. 3 and 4 reproduce \dot{O}_2 - T curves found for isolated mitochondria (Cossins and Bowler, 1987; Davison, 1971). In fact, mitochondria stimulated with ADP (Fig. 5; modified from Davison, 1971) display a curve similar to the dotted-curve (Fig. 4, aerobic

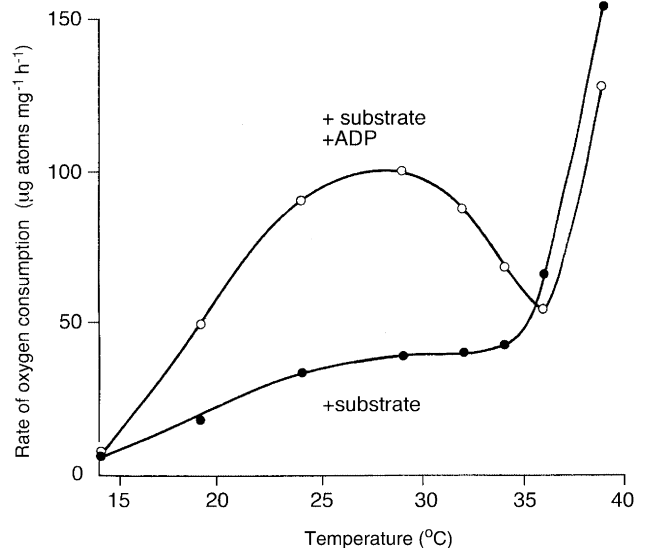


Fig. 5. Oxygen consumption curve as a function of temperature for isolated mitochondria of blowfly muscle (figure derived from data of Davison, 1971). Respiration in the presence of ADP, to stimulate respiration and substrate display a maximum of approximately 28–30 °C.

isometric contraction) and without ADP are similar to the dashed-curve (rest). Considering that ADP concentration is increased during exercise, the present in vivo results might reflect a phenomenon occurring at the mitochondrial level. Of course, some differences must be expected because the mitochondria come from different species but, the global behaviour probably remains the same (Cossins and Bowler, 1987). Further studies are necessary to confirm this hypothesis. The presence of a non-zero standard deviation for T values, explains the difference observed in the position of the peaks in Figs. 3 and 4. The comparison between rest and exercise, allows one to conclude that membrane phase T -transitions, i.e. T -induced conformational changes of molecules that may change light absorption and scattering parameters at a given critical T (Stubbs, 1983), are not sufficient to explain the observed \dot{O}_2 - T curves differences. If this were to be the case, phase T -transition-induced effects on \dot{O}_2 - T curves should be visible even during rest.

The isometric force developed in the present protocol was the same for all T values. Moreover, the exercise could be reasonably considered as aerobic (4% of the MVC). From this assumption, it follows that the efficiency of the aerobic metabolism, proportional to \dot{O}_2 at a given temperature, is T -dependent. The change in efficiency may be explained by at least two factors: (1) a recruitment of different fibres types for different T ; (2) a T -induced modulation of the enzymatic activity at the cellular level. Point (2) is compatible with the hypothesis that mitochondria T -dependent activity might be involved in the explanation of the observed phenomena.

Another possible interpretation of the present results would be that aerobic energy source yielding reactions have the same efficiency for all T , but that other anaerobic sources, (e.g. lactate production) compensate for the lack of energy to obtain the same mechanical workload. In fact, it has been demonstrated that during isotonic exercise at low temperature, lactate production is increased for a given workload compared to normothermia (Beelen and Sargeant, 1991; Masaru et al., 1992). It remains to be demonstrated that lactate production is actually increased in the present protocol.

5. Conclusions

In conclusion, the similarity of the shape of the \dot{O}_2 - T curves, (e.g. Figs. 3 and 4) to the \dot{O}_2 - T measurements obtained on mitochondria (Cossins and Bowler, 1987; Davison, 1971 and Fig. 5), allows one to hypothesise that probably a metabolic regulation and not a neuromuscular T -adaptation might explain the observed phenomena. Moreover, the observed behaviour of the \dot{O}_2 - T curves seems to hold for different species other than humans. In fact, many other animals, e.g. the anemone *Actinia equina* or the winkle *Littorina littorea*, during 'active' respiration, display \dot{O}_2 - T relationships having a sudden \dot{O}_2 increase for a given temperature (Newell and Northcroft, 1967). The present interpretation of the data remains compatible with the idea of an involvement of T -modulated activity of anaerobic metabolic pathways.

Acknowledgments

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