

Temperature Dependence of Human Gastrocnemius pH and High-Energy Phosphate Concentration by Noninvasive Techniques

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It is well established that ADP is an important regulator of the oxidative phosphorylation in the mitochondria. Thus, by means of noninvasive techniques it is demonstrated that the relationship between \dot{O}_2 consumption of the human gastrocnemius at rest and its temperature is likely determined by at least two factors: 1) the modulation of the rate of the chemical reactions imposed by the "physical" temperature-effect; 2) the influence of temperature-induced ADP concentration changes ($-0.83 \mu\text{M } ^\circ\text{C}^{-1}$) on oxidative phosphorylation. ADP was assessed by applying the temperature-corrected Lohmann equilibrium equation. PCr and ATP were found to increase, with decreasing temperature (-0.54 ± 0.05 and $-0.17 \pm \text{mM } ^\circ\text{C}^{-1}$, respectively), while pH varies following the α -stat hypothesis ($-0.016 \pm 0.001 \text{ pH } ^\circ\text{C}^{-1}$). These findings should be of value when dealing with muscle physiology in extreme environments or clinical applications of hypothermia. *Magn Reson Med* 43:611–614, 2000. © 2000 Wiley-Liss, Inc.

Key words: muscle; temperature; ³¹P-NMR spectroscopy; metabolism; oxygen consumption

Human skeletal muscle oxygen consumption (\dot{O}_2) is temperature (T) dependent. It has been demonstrated that a decrease of 10°C can reduce hand flexors resting \dot{O}_2 by a factor of ~2 (1). However, the determinants of the relationship between intramuscular \dot{O}_2 and T in humans have not yet been well established. In fact, many variables might influence \dot{O}_2 when changing T, i.e., thermodynamic phenomena, blood perfusion, enzymatic activity, the concentration of key metabolites, etc. For instance, it is well known that ADP concentration has a strong influence on the modulation of \dot{O}_2 (2). Moreover, it has been demonstrated in animals that T changes induce intracellular pH variations (3,4). Considering that ADP is correlated to pH through the Lohmann reaction, then a change in pH should also influence \dot{O}_2 . If this is the case, then phenomena other than the simple thermodynamic T-dependent slowing/increasing activity of the biochemical reactions will be necessary to explain \dot{O}_2 changes during temperature shifts.

The study of the determinants of the T- \dot{O}_2 relationship would be of interest in many domains and applications, e.g., environmental physiology, organ preservation, hypothermic cardiopulmonary bypass, etc. In this context, the aim of the present work was to study the T-dependence of the Lohmann reaction, i.e., to measure pH, ADP, ATP, and phosphocreatine concentration (PCr) in the human gastrocnemius at different T levels and to discuss the possible physiological implications on \dot{O}_2 . To our knowledge, these measurements have never been performed on humans.

MATERIALS AND METHODS

³¹P-NMR Spectroscopy

³¹P-NMR spectra were obtained on a hybrid system comprising a Picker (Picker International, Highland Heights, OH, USA) whole-body imaging system (1.5 T superconducting magnet, 90 cm bore) and a NMR console (Surrey Medical Imaging Systems, SMIS, Guildford, UK). A ³¹P 7-cm diameter RF surface coil was used. Shimming was performed manually on the proton signal using first-order correction. An RF pulse angle smaller than that required to maximize the in vivo ³¹P signal-to-noise ratio was selected in order to ensure that temperature-dependent changes in spin-lattice relaxation times would not introduce measurement artifacts during the acquisition of fully relaxed spectra. Practically, the pulse angle was reduced until the spectra were fully relaxed with a 16-sec repetition time (TR) reducing the depth profile of the region of interest to ~2 cm. Spectra were acquired continuously with a 16-sec TR. Acquisition parameters were: 100 μs for the RF pulse duration, 1024 points per free induction decay (FID), and a spectral width of 1000 Hz. A single spectrum consisted of the sum of 16 (TR = 16 sec) FIDs, corresponding to an acquisition time of 256 sec per spectrum. The FIDs were filtered with an exponential function (line broadening 3 Hz) to improve the signal-to-noise ratio.

Evaluation of the relative concentrations of PCr and ATP, and the measurement of the chemical shift of inorganic phosphate (Pi), PCr, α ATP, and β ATP was performed using a nonlinear least-square fitting method in the time domain (variable projection method, 5). Prior knowledge was incorporated for the β ATP triplet, imposing the same frequency splitting between the three peaks. The α ATP, β ATP, γ ATP peak areas *within* each multiplet were linked together using the following constants of proportionality: 1:1, 0.5:1:0.5, and 1:1. Considering that spectra were fully relaxed, the α ATP, β ATP, γ ATP peak areas were also linked *between* multiplets.

Peak areas were then corrected for the temperature effects on the magnetization (6,7) and expressed relative to

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37°C. In fact, the magnetization (M) of a set of N nuclei exposed to a magnetic field of strength B_0 is given (6) by:

$$M = \frac{N\gamma^2 \left(\frac{h}{2\pi}\right)^2 I(I+1)B_0}{3kT} \quad [1]$$

where κ is Boltzmann's constant, the temperature (Kelvin), γ the gyromagnetic ratio, h Planck's constant and I is the nuclear spin quantum number (in this case 1/2).

The following temperature-corrected algorithm was used to compute intracellular pH from Pi-PCr chemical shift (δ_{PiPCr}) (8):

$$\text{pH} = \frac{1979.5}{T + 273} - 5.4409 + 0.018567(T + 273) + \log \left(\frac{\delta_{\text{PiPCr}} - 3.280 + 0.003579T}{5.625 + 0.001888T - \delta_{\text{PiPCr}}} \right) \quad [2]$$

where T is expressed in °C.

The muscle volume detected by the ^{31}P RF coil is not necessarily equal among subjects (different adipose tissue thickness, etc.) and ATP and PCr values are usually expressed as a percent of a reference level (in arbitrary units). Thus, as adopted elsewhere (9), ATP and PCr values were normalized in order to obtain an ATP of 8.2 mM at 37°C for all subjects (10). It is noteworthy that ATP and PCr will be used only as a ratio for the estimation of ADP (see below); thus, the choice of an ATP at 37°C different from 8.2 mM will not influence the present results.

ADP values were calculated (11,12) using the following equations:

$$\text{ADP} = \frac{\text{ATP} \cdot \text{Cr}}{K'(T)\text{PCr}} \quad [3]$$

where $K'(T)$ is the apparent equilibrium constant at temperature T and Cr the free creatine concentration. $K'(T)$ was calculated by means of (12):

$$K'(38) = 10^{\Delta G'^{\circ}/(-2.303R(273.15+38))} \quad [4]$$

where $\Delta G'^{\circ} = -13.39 \text{ kJ mol}^{-1}$ is the standard apparent or transformed Gibbs energy of the reaction at 38°C, pH 7.0, free Mg^{2+} of 1 mM and ionic strength of 0.25. $R = 8.3145 \text{ J K}^{-1}$ is the gas constant and 2.303 is the conversion factor from \log_{10} to natural (ln). $K'(T)$ was then obtained using (12):

$$\log \left(\frac{K'(T)}{K'(38)} \right) = \frac{\Delta H'^{\circ}}{2.303R} \left(\frac{T - 38}{38T} \right) \quad [5]$$

where $\Delta H'^{\circ} = -11.93 \text{ kJ mol}^{-1}$ is the standard apparent or transformed enthalpy of the reaction for the range 5–38°C, at pH 7.0, free Mg^{2+} of 1 mM and ionic strength of 0.25.

Cr was calculated as:

$$\text{Cr} = \text{Cr}_{\text{tot}} - \text{PCr} \quad [6]$$

where $\text{Cr}_{\text{tot}} = 42.7 \text{ mM}$ is the total creatine concentration (17) at 37°C. Cr_{tot} was considered constant at all the temperatures (13).

Intramuscular Temperature Measurements

The ^{31}P -NMR RF coil was combined with a *noninvasive* NMR compatible zero-heat-flow based temperature (T) probe (Deep Body Thermometers Ltd., Little Eversden, Cambridge, UK) (14,15). The zero-heat-flow probe (ZHF) allows one to measure intramuscular T (16) at ~1.5 cm below the skin surface. T measurements were performed in this way in the same region where ^{31}P -NMR spectra were acquired. As explained elsewhere (14), the ZHF probe creates a uniform temperature distribution in the region of interest, reducing the size of the possible errors induced by tissue temperature gradients.

Subjects

The experiments were conducted on six (three females) healthy subjects age 39.7 ± 16.4 (SD) years, body weight $62.2 \pm 5.8 \text{ kg}$. They were aware of the aim of the study and of the related risks. The protocol was in accordance with the guidelines of the ethical committee of the Medical School of the University of Geneva.

Protocol

The subject was installed supine in the NMR magnet with the right calf placed over the combined ^{31}P -NMR/ZHF probe. The leg was fixed to the couch bed to avoid motion artifacts. The subject was covered with a woolen blanket for comfort and to maintain core temperature. The thermometer was switched on and allowed to stabilize for 20 min and the T monitored on a paper plotter (Servogor 120, BBC Goerz Metrawatt, UK) to easily follow the stabilization of the ZHF probe. At the end of this period, the right leg was uniformly covered with disposable ice bags and the protocol immediately started. ^{31}P -NMR spectra were acquired sequentially during the cooling procedure. To avoid global chemical shifts on ^{31}P -NMR spectra due to the small magnetic field generated by the ZHF probe, the thermometer was switched off during acquisition of spectra. In the intervals between spectra acquisition, the thermometer was switched on and the ZHF probe allowed to stabilize again. In this case, stabilization was reached in less than 8 min. T was measured before each spectra. The experiment was terminated after 3–3.5 hr, by which time the calf muscle had cooled to near 30°C in all subjects.

Statistics

Data are reported as means \pm SD and significance level for the linear regressions was set at $P < 0.05$.

RESULTS

In Fig. 1 are shown the pH values as a function of T for all subjects. pH is linearly correlated to T ($P < 0.05$) and the slope of the linear regression is $-0.016 \pm 0.001 \text{ pH } ^{\circ}\text{C}^{-1}$.

PCr and ATP values as a function of T are shown in Fig. 2 for all subjects. PCr and ATP are linearly correlated to T

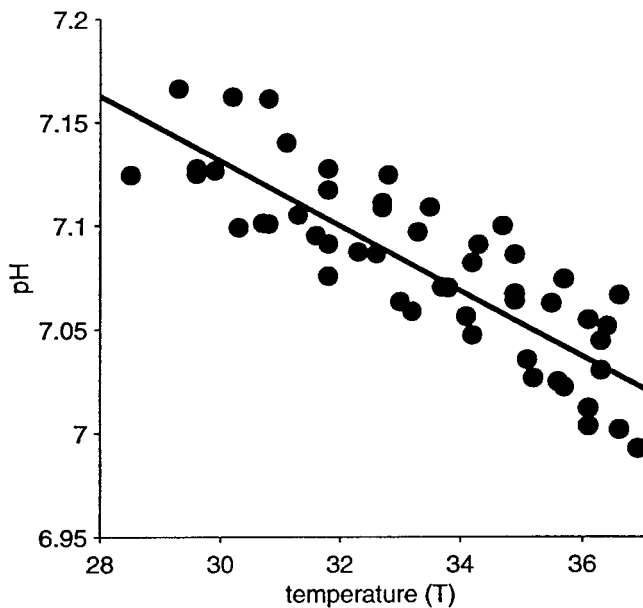


FIG. 1. Intracellular pH of human gastrocnemius ($n = 6$) as a function of temperature.

($P < 0.05$) and the slopes of the linear regressions, corresponding to the T-induced changes on PCr and ATP, are -0.54 ± 0.05 and -0.17 ± 0.01 mM $^{\circ}\text{C}^{-1}$, respectively. Pi did not change significantly (not shown).

In Fig. 3 are shown the ADP values as a function of T calculated using Eq. [3] and the above results. The thick line was evaluated assuming $\text{Cr}_{\text{tot}} = 42.7$ mM (10,13). The thin lines show the effect of assuming different Cr_{tot} at 37°C. The Cr_{tot} values go from 40 mM (bottom line) to 50 mM (top line) by step of 1 mM. ADP decreases nearly

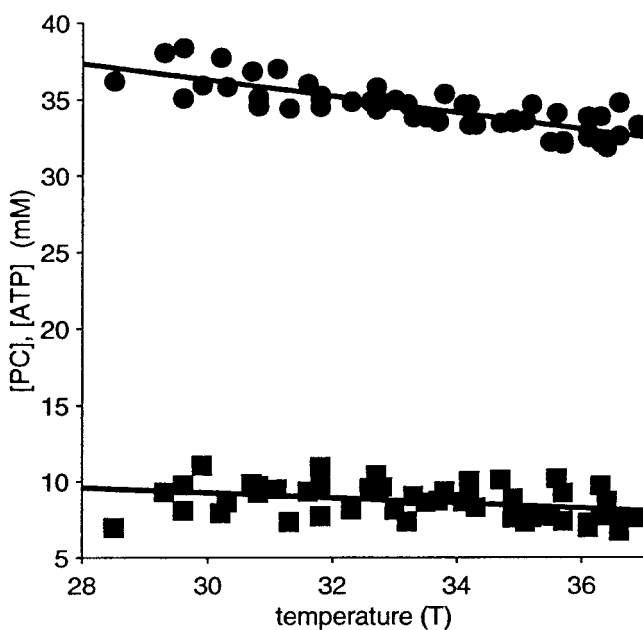


FIG. 2. PCr (circle) and ATP (square) in human gastrocnemius as a function of temperature.

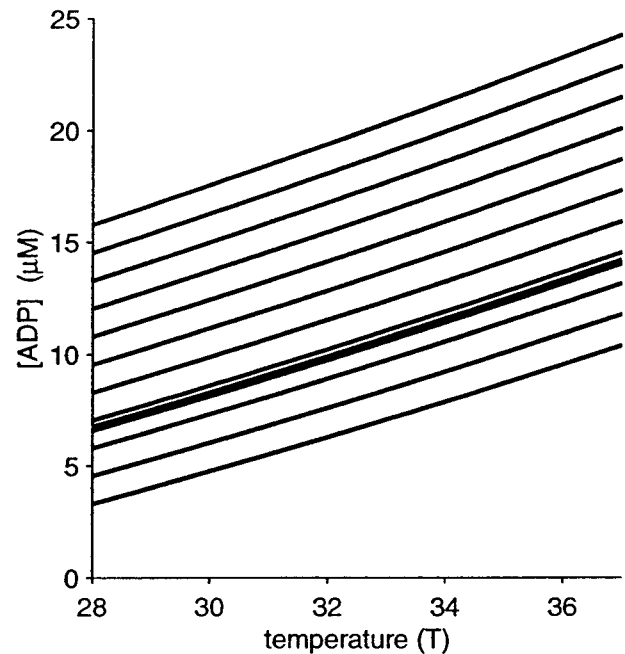


FIG. 3. ADP concentration in human gastrocnemius as a function of temperature. The values were calculated using a temperature-corrected Lohmann equilibrium equation. The thin lines were calculated for Cr_{tot} from 40 mM (bottom) to 50 mM (top), by step of 1 mM. The thick line corresponds to $\text{Cr}_{\text{tot}} = 42.7$ mM.

linearly for all the lines ($\sim 0.83 \mu\text{M } ^{\circ}\text{C}^{-1}$), with decreasing temperature.

DISCUSSION

Intracellular acid base regulation is usually considered essential for the maintenance of the chemical environment and for the optimal function of the enzymatic reactions. In fact, Reeves (17) generalized this concept and advanced the hypothesis that vascular and intracellular pH are not regulated per se, but it is the ratio of unprotonated to total protein histidine imidazole the primary regulated variable. This was called the α -stat hypothesis. α -stat regulation should provide a constant protein net charge, important for preserving structural and functional properties and optimal reaction rates of temperature-sensitive enzymes and other proteins (4). The present measured value of -0.016 ± 0.001 pH $^{\circ}\text{C}^{-1}$ is compatible with the α -stat theory (3). In fact, enzyme function is better preserved, for nearly all enzymes, with a value of -0.016 pH $^{\circ}\text{C}^{-1}$ (18).

In the present study, Pi was not found to decrease with decreasing temperature, as in soleus cat muscle (19). In fact, the authors measured, by ^{31}P -NMR spectroscopy, Pi values of 8.5 ± 5.5 and 6.0 ± 1.0 mM at 30 and 22°C, respectively. However, it must be noted that in the cited study (19), the hypothesis was made that ATP is the same at all temperatures. Pi was calculated consequently using this ATP-based spectra calibration procedure. If one calculates ATP at 22°C, based on the ATP change per $^{\circ}\text{C}$ found in the present study (-0.17 ± 0.01 mM $^{\circ}\text{C}^{-1}$), one finds for the 30 and 22°C range a Pi shift from 8.5 ± 5.5 to 7.63 mM, only. Considering the large variability of the Pi values

and the fact that cat and human muscles may have different functional properties, the T-dependent Pi changes may be neglected, as in the present study. Furthermore, specific measurements are needed, even though the main conclusion of the present study should not be affected.

Increased pH, ATP, and PCr (see Fig. 2) with decreasing temperature influence the Lohmann equilibrium. The same changes in pH and high energy phosphates concentration were observed also in animals (7,19). This results in a decreased ADP at low T (Fig. 3). As is well known, ADP modulates \dot{O}_2 (2) and, in this case, its decrease should explain part of the observed \dot{O}_2 change during muscle cooling (1), probably amplifying the pure thermodynamic T-effect on \dot{O}_2 . A simple estimate of the magnitude of the change in oxidative metabolism may be obtained using Chance's transfer function for metabolic control of oxidative phosphorylation ($V/V_{\max} = \{1 + K_m/ADP\}^{-1}$ and $K_m = 20 \mu\text{M}$) (20). In this case, a change of ADP from 7 to 14 μM produces a variation of V/V_{\max} from ~ 0.26 to ~ 0.41 (slightly different K_m give analogous results). Hence, the effect of ADP on \dot{O}_2 is, in principle, not negligible. From Fig. 3 it can also be seen that a different level of Cr_{tot} at 37°C does not influence this conclusion. In fact, the slope of the lines are practically the same ($\sim 0.83 \mu\text{M } ^\circ\text{C}^{-1}$) for all Cr_{tot} values. From the same figure it can be inferred that to eliminate the T-effect on ADP, intracellular Cr_{tot} should increase monotonically from 42.7 mM to ~ 48 mM, when shifting from 37°C to 28°C (or a change of ~ 5.3 mM, starting from any given Cr_{tot} concentration at 37°C). To our knowledge, this Cr_{tot} increase has never been observed.

In conclusion, the human gastrocnemius \dot{O}_2 -T relationship is probably determined by at least two factors: 1) the modulation of the speed of the chemical reactions imposed by the "physical" T-effect, and 2) the influence of T-induced ADP changes on oxidative phosphorylation.

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REFERENCES

- Binzoni T, Springett R, Dalton JCP, Delpy D. A new combined deep-body-temperature/NIRS probe for non invasive metabolic measurements on human skeletal muscle. *Adv Med Exp Biol* 1999;471:623-629.
- Brown C. Control of respiration and ATP synthesis in mammalian mitochondria and cells. *Biochem J* 1992;284:1-13.
- Hitzig BM, Perng W-C, Burt T, Okunieff P, Johnson DC. $^1\text{H-NMR}$ measurement of fractional dissociation of imidazole in intact animals. *Am J Physiol* 1994;266 (Regul Integr Comp Physiol 35:R1008-R1015).
- Rahn H, Prakash O, editors. Acid base regulation and body temperature. Dordrecht: Martinus Nijhoff; 1985. p 161.
- van der Veen JWC, de Beer R, Luyten PR, van Ormondt D. Accurate quantification of in vivo ^{31}P NMR signals using the variable projection method and prior knowledge. *Magn Reson Med* 1988;6:92-98.
- Abragam A. The principles of nuclear magnetism. London: Oxford University Press; 1961.
- Binzoni T, Ferretti G, Barbalat F, Cerretelli P. Energetics of resting anaerobic frog gastrocnemius at different temperatures by $^{31}\text{P-NMR}$. *Respir Physiol* 1990;82:137-148.
- Kost GJ. pH standardization for phosphorus-31 magnetic resonance heart spectroscopy at different temperatures. *Magn Reson Med* 1990; 14:496-506.
- Jeneson JAL, Westerhoff HV, Brown TR, van Echteld CJA, Berger R. Quasi-linear relationship between Gibbs free energy of ATP hydrolysis and power output in human forearm muscle. *Am J Physiol* 1995;268 (Cell Physiol 37:C1474-C1484).
- Harris RC, Hultman E, Nordesjo L-O. Glycogen, glycolytic intermediates and high-energy phosphates determined in biopsy samples of musculus quadriceps femoris of man at rest. Methods and variance of values. *Scand J Clin Lab Invest* 1974;33:109-120.
- Lawson JWR, Veech RL. Effects of pH and free Mg^{2+} on the K_{eq} of the creatine kinase reaction and other phosphate hydrolyses and phosphate transfer reactions. *J Biol Chem* 1979;254:6528-6537.
- Teague WE, Dobson GP. Effect of temperature on the creatine kinase equilibrium. *J Biol Chem* 1992;267:14084-14093.
- Goudemant J-F, Vander Elst L, Dupont B, Van Haverbeke Y, Muller RN. pH and temperature effects on kinetics of creatine kinase in aqueous solution and in isovolumetric perfused heart. A ^{31}P nuclear magnetization transfer study. *NMR Biomed* 1994;7:101-110.
- Fox RH, Solman AJ. A new technique for monitoring the deep body temperature in man from the intact skin surface. *J Physiol* 1971;212: 8P-10P.
- Binzoni T, Hiltbrand E, Terrier F, Cerretelli P, Delpy D. Temperature dependence of human gastrocnemius pH and high energy phosphate concentrations by non-invasive techniques. In: Proc of the 7th Annual Meeting of ISMRM, Philadelphia, 1999. p 1520.
- Togawa T, Nemoto T, Yamazaki T, Kobayashi T. A modified internal temperature measurement device. *Med Biol Eng* 1976;14:361-364.
- Reeves RB. An imidazole alphastat hypothesis for vertebrate acid-base regulation. *Respir Physiol* 1972;14:219-236.
- Heisler N. Comparative aspects of acid-base regulation. In: Heisler N, editor. Acid base regulation in animals. Amsterdam: Elsevier; 1986. p 397-449.
- McFarland EW, Kushmerick MJ, Moerland TS. Activity of creatine kinase in contracting mammalian muscle of uniform type. *Biophys J* 1994;67:1912-1924.
- Chance B, Leigh JS, Clark BJ Jr, Maris J, Kent J, Nioka J, Smith D. Control of oxidative metabolism and oxygen delivery in human skeletal muscle: a steady-state analysis of the work/energy cost transfer function. *Proc Natl Acad Sci USA* 1985;82:8384-8388.