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Noninvasive estimation of temperature and pH in human lower leg muscles using ^{1}H nuclear magnetic resonance spectroscopy

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Abstract. The temperature and pH of human lower leg muscles were estimated noninvasively using ^{1}H-NMR spectroscopy at 3.0 and 1.5 T on five normal volunteers (21M, 24M, 27M, 34M, 47M). The chemical shifts of water and imidazole protons relative to cholines ($-N^+CH_3$) or creatines ($-CH_3$) could be used as the temperature and pH probes, respectively [1–4]. Using the chemical shift, estimated values of the temperature in gastrocnemius (GAS; shell region) and soleus muscles (SOL; core region) under ambient temperature (21–25°C) were 33.6 ± 0.4 and 35.3 ± 0.4°C (mean ± SE), respectively (significantly different; $P < 0.01$). The values of pH in these muscles were estimated to be 6.97 ± 0.01 and 6.96 ± 0.02, respectively. Alternation of the surface temperature of the lower leg from 40 to 10°C significantly changed the temperature in GAS ($P < 0.0001$) from 35.8 ± 0.4 to 26.2 ± 1.2°C and the pH in GAS rose from 6.95 ± 0.01 to 7.01 ± 0.01 ($P < 0.01$). However, the values of pH and temperature in SOL were not significantly affected by this maneuver. These results indicate that the pH in GAS was moderately changed by muscle temperature ($r = -0.59$, $P < 0.01$), and its change was estimated to be -0.005 pH units/°C.

Keywords: Magnetic resonance spectroscopy, temperature, pH, human lower leg muscles

1. Introduction

The intracellular pH of tissues and organs has been estimated using *in vivo* and *ex vivo* ^{1}H- and ^{31}P-NMR spectroscopy [3,5–9]. Although pH is known to be changed with temperature [4,10–12], this change in human tissues under physiological conditions has not been measured because the noninvasive measurement of the human body temperature in deep regions is not well established. Therefore, using ^{1}H magnetic resonance spectroscopy, we tried to measure the temperature and pH of human tissues simultaneously. NMR techniques for temperature measurement are based mainly on the temperature dependence of the NMR relaxation time, diffusion coefficient or chemical shift [1,2,13–24]. An accuracy of better than ±1°C is desirable for applying these techniques to estimate the temperature in the human body under physiological conditions. Corbett et al. [1] and Cady et al. [2] have reported novel techniques to estimate

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the absolute temperature of the human brain under physiological conditions. They used the chemical shift difference between the water and NAA as the temperature probe. We used the same approach to estimate temperature in human lower leg muscles, in which the chemical shift difference between water and choline or creatine was used as the probe. Using *in vivo* and *in vitro* ^{1}H-NMR spectroscopy, metabolites in the muscles such as creatines (creatine, phosphocreatine, creatinine), cholines (choline, phosphorylcholine) and carnosine could be simultaneously analyzed [3,6–9,25]. The pH in the muscle could be estimated based on the pH dependence of the chemical shift of imidazole protons in carnosine [3,4,6–9]. Therefore, the temperature and pH could be estimated by ^{1}H-NMR spectroscopy in the human muscles simultaneously, and the correlation between the temperature and pH could be assessed.

2. Materials and methods

2.1. Spectroscopy and data processing

^{1}H-NMR spectroscopy was performed at 3.0 and 1.5 T (General Electric: Signa Horizon LX 3T and LX 1.5 T) on five volunteers (21M, 24M, 27M, 34M, 47M) and on excised pig skeletal muscles with point-resolved spectroscopy (PRESS) localization. The experimental protocols were approved by the local Ethics Committee of Iwate Medical University. The NMR measurements were performed after obtaining appropriate consent forms signed by the volunteers. The spectrum of a human leg was obtained during cooling and warming from the surface of the lower leg as well as under ambient temperature (21 to 25°C). The skin surface temperatures during cooling and warming were about 10 and 40°C, respectively. Acquisition parameters were as follows: at 3.0 T; 3000 ms repetition time, 30–144 ms echo time, 2 K data size, 2500 Hz spectral width, 8 to 256 acquisitions: at 1.5 T; 1500 ms repetition time, 30–288 ms echo time, 2 K data size, 2500 Hz spectral width, 16 to 512 acquisitions. The voxel sizes were 25 to 40 ml for human lower leg muscles and 8 ml for pig skeletal muscles. The raw data were transferred from Signa to a PC and processed on the PC (zero filling, apodization, FFT, and peak fitting). Typical ^{1}H NMR spectra of human lower leg muscles at 3.0 T are shown in Fig. 1.

The spectrum of Fig. 1(a) was obtained without water decoupling to estimate the temperature and that of Fig. 1(b) with water decoupling to estimate the pH. The NMR spectra were analyzed by an automatic curve-fitting procedure and broken down into the components of the Lorentzian peaks (GRAMS/32; Galactic Industries Corp., Salem, NH, USA). Scheffe's test was used for post hoc multiple comparisons between different pairs of means after one-way ANOVA indicated significant differences. The correlation between the temperature and pH was examined by Pearson's test.

2.2. Calibration curve to estimate temperature

Calibration curves were obtained using excised pig skeletal muscles at different temperatures. The temperature of the muscles was monitored by a copper–constantan thermocouple (the absolute accuracy: ± 0.1°C). The temperature drift in the muscles during measurements was less than 1.0°C. Calibration curves obtained are shown in Fig. 2.

The difference between the chemical shifts for choline or creatine and water showed a linear function of increasing temperature from 20 to 40°C (slope: -0.00971 and -0.00999 ppm/°C, r: -0.996 and -0.996 for choline and creatine, respectively). The precision of this method was also examined using pig skeletal muscles, where the temperature drift in the muscle during measurements was less than 0.1°C. The standard deviation of the estimated temperature was about ± 0.1°C.

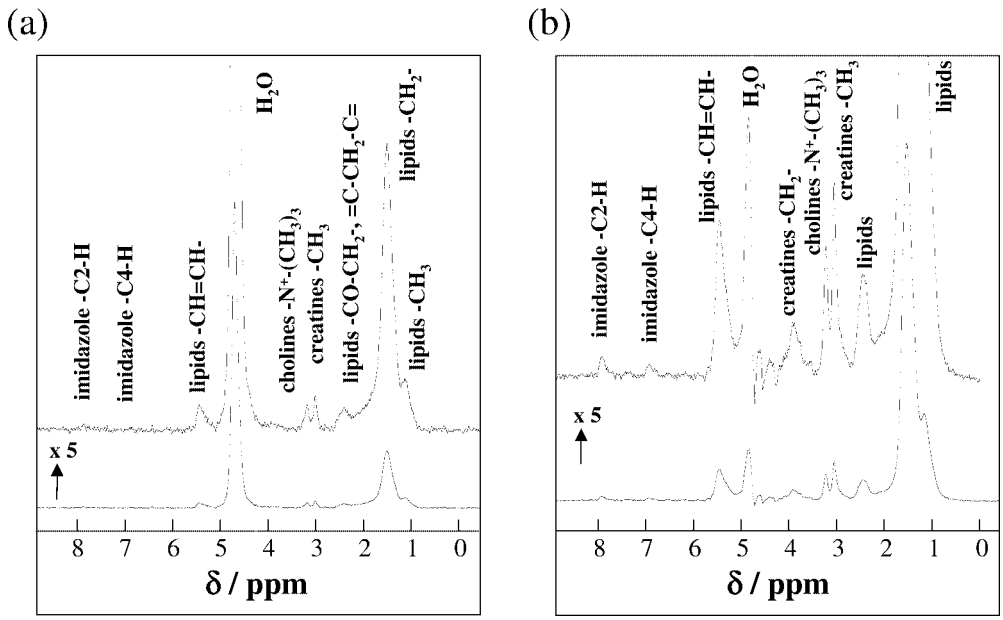

Fig. 1. Typical ^{1}H-NMR spectrum of human lower leg muscles (gastrocnemius) at 3.0 T. Spectrum (a) was obtained without water suppression and (b) with water suppression. The chemical shift was calculated based on the shift of creatine (3.0244 ppm from DSS [dimethylsilapentane sodium sulfonate] methyl signal). Acquisition parameters were as follows: 3000 ms repetition time, 144 ms echo time, 2500 Hz spectral width, 8 for (a) and 256 for (b) acquisitions. Accumulation over a long time is necessary to detect imidazole signals.

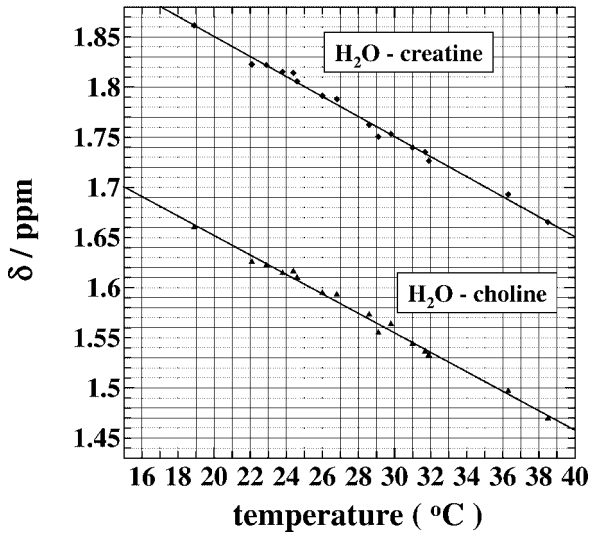

Fig. 2. Calibration curves for temperature estimation. These curves were obtained using excised pig skeletal muscles at different temperatures. The temperature of the muscle was monitored by a copper–constantan thermocouple. The drift in the muscle temperature during measurements was less than 1.0°C. The ordinate is the chemical shift difference between water and choline or creatine signals. These chemical shift differences showed a linear function of increasing temperature from 20 to 40°C (slope: −0.00971 and −0.00999 ppm/°C, r: −0.996 and −0.996 for choline and creatine, respectively).

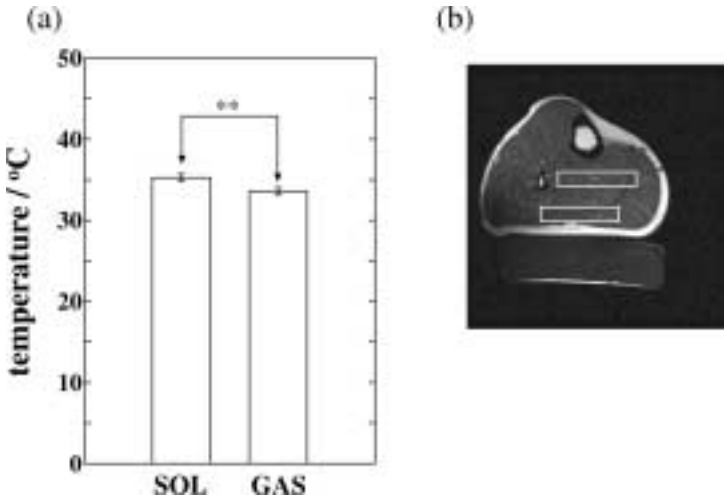

Fig. 3. (a): Estimated values of the temperature in human lower leg muscles under ambient temperature (21 to 25°C). (b): The region of interest. Values are expressed as mean plus/minus SE. $^{**}P < 0.01$. Abbreviations used: SOL, soleus muscles; GAS, gastrocnemius.

2.3. Estimation of pH

Intracellular pH was calculated from the chemical shift of the imidazole proton based on the equation

$$\text{pH} = 7.251 - 0.0154 \times T + \log[(5.537 - \delta)/(\delta - 4.618)],$$

where δ equals the chemical shift of the imidazole (C2 proton) signal relative to creatine and T is the temperature in °C [4,6–9].

3. Results

3.1. Estimated temperature of volunteers

Estimated values of the temperature in the gastrocnemius (GAS; shell region) and soleus muscles (SOL; core region) under ambient temperature (21 to 25°C) were 33.6±0.4 and 35.3±0.4°C (mean±SE), respectively (significantly different; $P < 0.01$) (Fig. 3).

Alternation of the surface temperature of the lower leg from 40 to 10°C significantly changed the temperature in the GAS ($P < 0.0001$) from 35.8 ± 0.4 to 26.2 ± 1.2°C, but induced no significant change in the SOL (Fig. 4).

3.2. Estimated pH of volunteers

The values of pH in the GAS and SOL under ambient temperature were estimated to be 6.97 ± 0.01 and 6.96 ± 0.02, respectively. Upon cooling the surface temperature of the lower leg from 40 to 10°C, the pH rose significantly in the GAS ($P < 0.01$) from 6.95 ± 0.01 to 7.01 ± 0.01, but not in the SOL (Fig. 5).

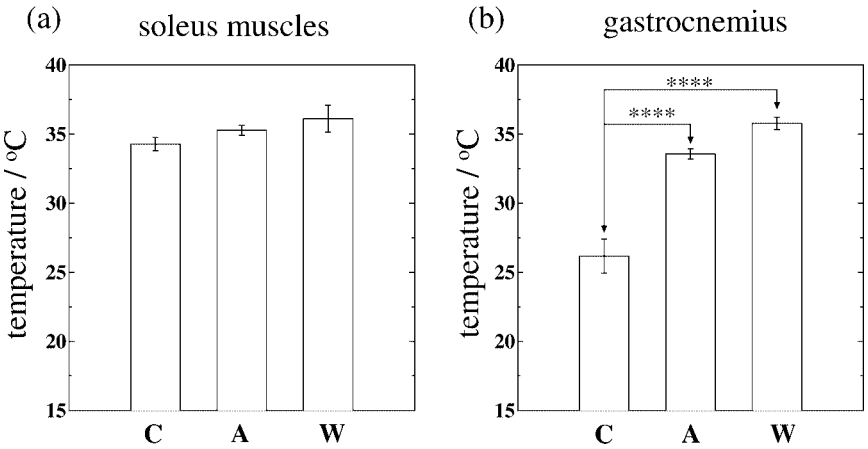

Fig. 4. Estimated values of the temperature in human lower leg muscles during cooling and warming, and at ambient temperature. (a) Temperature in the soleus muscles. (b) Temperature in the gastrocnemius. Values are expressed as mean plus/minus SE. $^{****}P < 0.0001$. Abbreviations used: C, cooling; A, ambient temperature; W, warming.

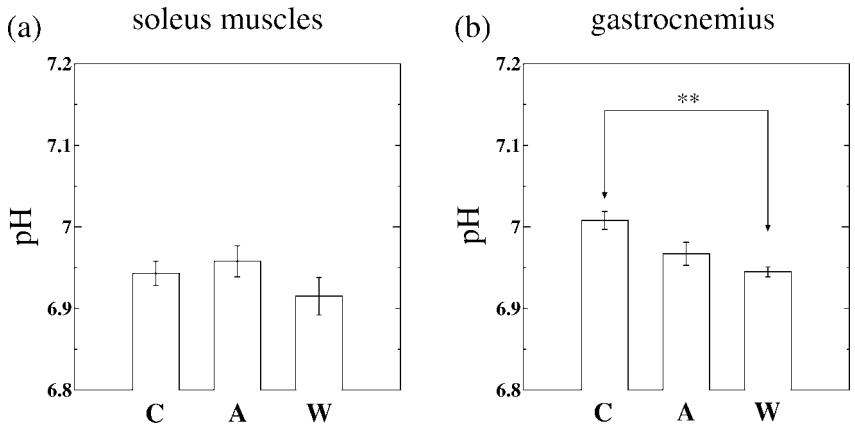

Fig. 5. Estimated values of pH in human lower leg muscles during cooling and warming, and at ambient temperature. Values are expressed as mean plus/minus SE. $^{**}P < 0.01$. Abbreviations used: C, cooling; A, ambient temperature; W, warming.

3.3. Correlation between pH and temperature

The pH in the GAS significantly correlated with the temperature ($r = -0.59$, $P < 0.01$) (Fig. 6). The slope calculated was -0.005 pH units/°C.

4. Discussion and conclusion

^{1}H NMR spectroscopy provided a noninvasive method of estimating the temperature and pH in human lower leg muscles. The temperature in the GAS (shell region) was lower by about 1.7°C than that in the SOL (core region) under ambient temperature. The values of the temperature and pH in the GAS were changed by cooling from the surface of the lower leg, but not in the SOL. The pH in the GAS correlated with the temperature and its slope was estimated to be -0.005 pH units/°C. This slope is smaller than

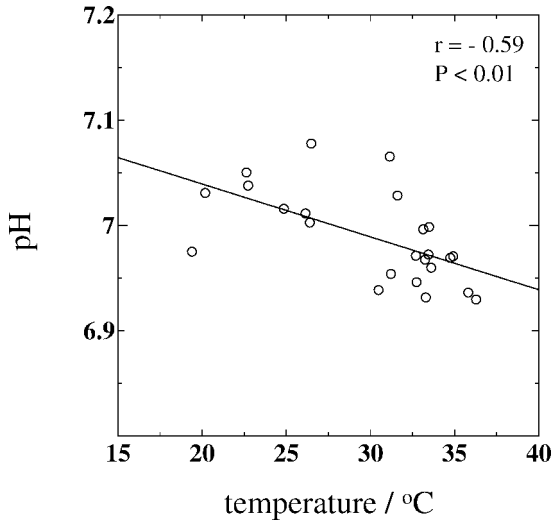

Fig. 6. Correlation plots of the temperature and pH examined with Pearson's test. The slope is -0.005 pH units/°C ($r = -0.59$, $P < 0.01$).

that of the previous *in vitro* study, where the slope calculated was -0.015 to -0.020 pH units/°C [10, 26]. Hitzig et al. [4] reported a comparable value of -0.015 pH units/°C for intact amphibian skeletal muscles. Intracellular acid–base regulation is important for maintenance of the chemical environment necessary for the optimal function of enzymes and reactions [7,8,26]. Reevs [26] has advanced the idea that arterial and intracellular pH are not regulated per se but the ratio of unprotonated to total protein histidine imidazole (fractional dissociation of imidazole, or α-imidazole) is the primary regulated variable. The imidazole group of histidine is the only hydrophilic amino acid that can reversibly bind a proton within the physiological pH range. An expression for the fractional dissociation of imidazole can be derived from the following equations:

$$\text{Im H}^+ = \text{Im} + \text{H}^+,$$

$$K = [\text{Im}][\text{H}^+]/[\text{Im H}^+],$$

$$\alpha\text{-imidazole} = [\text{Im}]/([\text{Im}] + [\text{Im H}^+]),$$

where Im is the imidazole group.

We could also estimate α-imidazole from the chemical shift of the imidazole proton. The α-imidazole in the GAS significantly correlated with the temperature ($r = 0.84$, $P < 0.00001$). Estimated values of α-imidazole were about 0.65 and 0.55 at 35 and 20°C, respectively. The alphastat hypothesis states that intracellular acid-base status is regulated to maintain constancy of the fractional dissociation of intracellular protein and enzyme imidazole-histidine (α-imidazole) [4,10,26]. However, α-imidazole in our experiments varied with muscle temperature. Unfortunately, we could not explain the reason for the difference between our human data and others. Further studies should be necessary in the future.

In summary, we described a method using ^{1}H NMR spectroscopy for estimating the temperature and pH in human muscles noninvasively and indicated that pH in the muscles correlates with muscle temperature. Additionally, we demonstrated that the electronic structure of the imidazole ring varies with temperature.

Acknowledgements

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