Fiber type composition affects resting ATP turnover estimated by NIRS in human ischemic muscle

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Abstract

The purpose of this study was to investigate effects of fiber type composition on resting ATP turnover at ischemic rest in human gastrocnemius (GC), tibialis anterior (TA) and soleus (SL) muscles using continuous-wave near-infrared spectroscopy (NIRS). Fiber type composition of the muscles was evaluated by histochemical analysis of muscle biopsies. Type I fiber occupied 54.0 ± 5.3, 62.2 ± 2.6 ($p < 0.05$) and 87.7 ± 3.3 % ($p < 0.01$, $p < 0.05$) of total fiber cross-section area in GC, TA and SL, respectively. Initial rate of decrease in muscle oxygenation by NIRS during ischemia was converted to resting ATP turnover rate using by muscle oxygen store at rest and a phosphate-to-oxygen ratio of 5.5. Resting ATP turnover rate was also corrected for muscle temperature decrease during the ischemic period. It was 0.281 ± 0.046, 0.277 ± 0.022 and 0.366 ± 0.046 mmol・(kg wet wt)$^{-1}$・min^{-1} in GC, TA and SL, respectively. Resting ATP turnover rate correlated with the relative type I fiber area ($r = 0.748$, $p < 0.01$) for the pooled data of all three muscles. It is suggested that the relative type I fiber area is an important factor determining resting metabolic rate in human muscles *in vivo*.

Key words: NIRS, % type I fibers, resting metabolic rate, ischemia

Introduction

It has been demonstrated that skeletal muscle characteristics are important factors for maintaining human health. For example, muscle fiber composition and capillarization correlate significantly with some important life-style illness[1]. Helge et al.[2] showed that there was a significant inversely relationship between the percent body fat and the percentage of type I fibers in a leg muscle. Tikkanen et al.[3,4] suggested that a higher percentage of type I fibers may be one factor having a beneficial effect on serum high-density lipoprotein cholesterol concentration. Skeletal muscles account for some 20 % of total energy expenditure at rest and muscle fiber type composition has a significant effect on daily energy expenditure of

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Abbreviations used: GC, gastrocnemius; TA, tibialis anterior; SL, soleus; NIRS, continuous-wave near-infrared spectroscopy; Oxy(Hb+Mb), oxyhemoglobin and oxymyoglobin of muscles; PCr, muscle phosphocreatine

humans[5]. However, very little is known about the relationship between muscle fiber type composition and ATP turnover at rest.

Skeletal muscles are comprised of muscle fibers that can be divided into two major types, type I and II. Type I fibers are rich in mitochondria as well as capillaries and are superior in aerobic energy metabolism compared with type II fibers[6]. It has been demonstrated that animal muscles, in which oxidative fibers (SO and FOG) are more abundant, have higher resting oxygen uptake compared with muscles prevailed by glycolytic (FG)[6,7,8,9]. However, it is unclear if such a relationship exists in human muscles.

The continuous-wave near-infrared spectroscopy (NIRS) and ^{31}P-magnetic-resonance spectroscopy (^{31}P-MRS) have been commonly used to study muscle oxygenation and energy metabolism, respectively. Under ischemic conditions, these two methods can estimate muscle ATP turnover at rest. Oxyhemoglobin and oxymyoglobin of muscles [oxy(Hb+Mb)] starts to decline immediately after muscle blood flow is arrested, since oxygen is used for aerobic ATP resynthesis[10]. It has been suggested that the initial rate of decrease in oxy(Hb+Mb) is proportional to muscle ATP turnover at rest[10]. After depletion of intramuscular oxygen stores, muscle phosphocreatine (PCr) also starts to decrease as it is used for ATP resynthesis[11].

The present study is to elucidate ATP turnover rate estimated by NIRS in human skeletal muscle at rest as a function of fiber type composition.

Methods

Subjects. Six healthy male subjects (age 25-36 yr, height 170-191 cm and weight 61-90 kg) gave their informed consent to take part in this study that was approved by the local Ethics Committee. None of the subjects participated in any specific training program.

Experimental design. Experiments were conducted at a room temperature of 23 °C. Subjects rested for approximately 30 min in the sitting position before the start of the experiment. Ischemia in lower leg muscles was induced by inflating a pneumatic 16-cm wide cuff on the thigh to a pressure of > 300 mmHg. The experiment always consisted of 10-min rest, 20-min muscle ischemia followed by 12-min recovery. This was repeated in NIRS experiment on gastrocnemius, tibialis anterior and soleus muscles. All three experiments were completed with five subjects over a three-week period. Subsequently, muscle biopsies were obtained from the same positions where the measuring probe of NIRS had been placed. Intramuscular temperature measurements were performed in a separate set of experiments on three subjects using the same protocol (one of three subjects participated in only temperature experiment).

NIRS measurements. The continuous-wave near-infrared (NIRS) spectrometer (HEO-100, Omron, Tokyo, Japan), consisting of the NIRS probe and computerized control system, was used to monitor both changes of oxy(Hb+Mb) and total hemoglobin (Hb) reflecting muscle oxygenation and blood volume. The probe contained a light source (760 nm and 840 nm) and an optical detector (photodiode), with 3.5 cm source-detector distance. For GC and TA, the probe was placed on the mid-portions of lateral GC and TA, respectively. For SL, the probe covered the muscle 2 - 3 cm distal from where medial and lateral heads of GC join the Achilles tendon. The NIRS signals were recorded every 0.5 sec throughout the protocol and averaged over 10 sec intervals. The NIRS signals were expressed as percentage oxygenation relative to the overall change from the rest to the minimum level observed during ischemia.

Resting ATP turnover rate. Data on NIRS measurement, the initial rate of decrease in oxy(Hb+Mb) during the first one minute of ischemia [$R_{(deoxy)}$] was converted to ATP turnover rate used by muscle oxygen store at rest [$M_{(oxy)}$] and a phosphate-to-oxygen (P/O_2) ratio[13] from a following equation:

$$\text{ATP turnover rate} = M_{(oxy)} \cdot R_{(deoxy)} \cdot P/O_2 \text{ ratio}$$

Muscle oxygen store at rest [mmol·(kg wet wt)$^{-1}$] was estimated from myoglobin content in type I and II fiber[12], and capillary-to-fiber ratio of GC[14], TA[15] and SL[16] previously reported. The capillary diameter was assumed to be 6 μm and half-saturated blood to be containing 0.1 ml O_2·(ml blood)$^{-1}$, respcetively[11]. Muscle oxygen consumption at rest was calculated to multiply muscle oxygen store by the initial rate of decline in oxy(Hb+Mb). This value was converted to resting ATP turnover rate by P/O_2 ratio = 5.5[13].

Temperature correction. Occlusion of muscle blood supply induces a decrease in intramuscular temperature during ischemia[17]. This has not been

evaluated in previous studies on ischemia at rest. A separate set of experiments was performed on three subjects using the needle thermistor (DK-8, Ellab, Copenhagen, Denmark). The measurements were made separately in each of the three muscles. The probe (0.8 mm diameter needle) was introduced percutaneously about 1 - 2 cm into the muscle and temperature was recorded every 30 sec. The protocol and ambient temperature was the same as that used for NIRS and ^{31}P-MRS measurements. The mean decrease during the course of the experiment was 0.4 ± 0.2, 1.0 ± 0.3 and 0.8 ± 0.3 °C in GC, TA and SL, respectively. These data were used to correct the observed ATP turnover rates expected at 37 °C, assuming a Q_{10} of 2.2 in the Arrhenius equation as follow:

ATP turnover rate (37 °C) =

ATP turnover (measured)·2.2·$\log_{10}$(37 - T)

where T is mean temperature during ischemic interval.

Histochemical analysis. Muscle samples were obtained from GC, TA and SL in each subject, using a Bergström-type needle[18] with suction. The tissue samples were mounted in imbedding medium and frozen rapidly in isopenthane precooled with liquid nitrogen. Transverse serial sections (10 μm) were cut by a cryostat at -20 °C and placed on a slide glass for myofibrilar ATPase staining[19]. The sections were preincubated at a pH of 10.3, 4.6 and 4.3, and incubated for myofibrilar ATPase reaction at pH of 9.4. Fiber types were classified in the light microscope as type I, IIA and IIX from a mean value of 226 (range 131 - 446) fibers in each biopsy by use of an image analysis system (TAMA, Denmark).

Statistical analysis. All data are expressed as mean (± SEM). Analysis of the data was performed using one-way analysis of variance (ANOVA). In the case of a significant F value, Scheffe test for multiple comparisons was used to assess differences between specific group means. Relationships between valuables were studied by regression analysis. Significance was accepted at the 5 % level.

Table 1. Fibre type composition in gastrocnemius (GC), tibialis anterior (TA) and soleus (SL) muscles.

Muscle	Type I fibres (%)	Type IIA fibres (%)	Type IIX fibres (%)
GC	54.0 (5.3)	29.5 (4.1)	16.4 (5.6)
TA	62.2 (2.6) ++	30.6 (4.6)	6.4 (3.2) ++
SL	87.7 (3.3) *++	10.3 (2.8) ++	2.0 (1.2) ++

Vaules are means (SEM). * ($p<0.05$) and++ ($p<0.01$) denote significant difference from TA and GC, respectively.

Results

Fiber type composition. Data on fiber type composition of GC, TA and SL muscles are presented in Table 1. The relative content of type I fibers (% type I fibers) was the highest in SL and the lowest in GC. Similar findings have been reported in a human autopsy study by Johnson et al[20].

Muscle oxygenation during ischemia. Data on

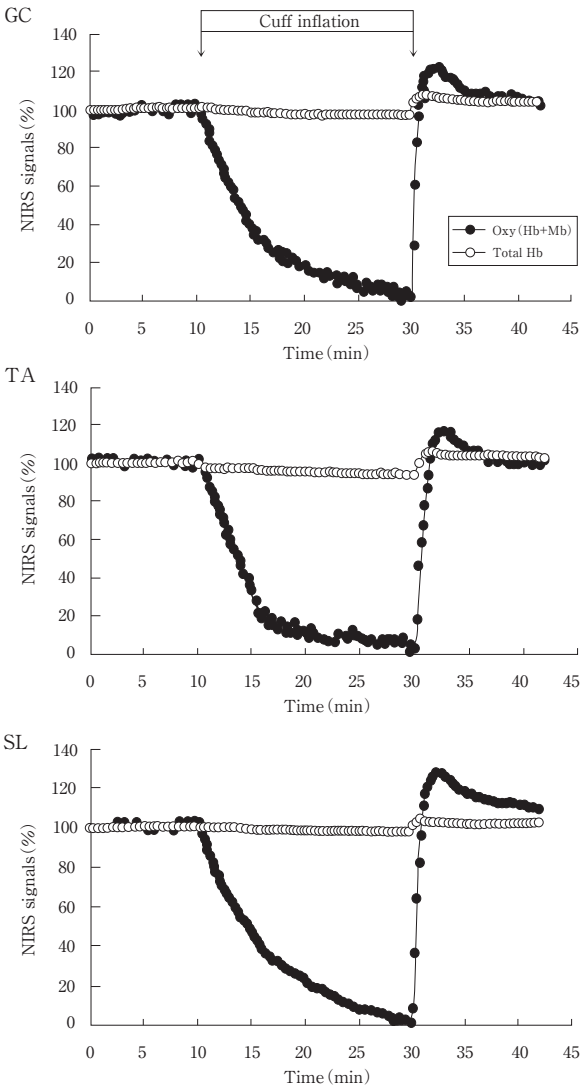

Fig. 1 Changes in muscle oxygenation [oxy(Hb+Mb)] and total Hb during the experimental protocol (10-min rest, 20-min ischemia and 12-min recovery) measured by near-infrared spectroscopy.

oxy(Hb+Mb) and total Hb during ischemia are presented in Fig. 1. The total Hb did not change during ischemia. But it tended to overshoot during recovery phase after ischemia. The latter phenomenon which is probably due to hyperemia after ischemia was especially pronounced in TA. Oxy(Hb+Mb) began to decrease immediately after cuff inflation, initially with linear kinetics and then tailing off after 4 - 6 min. The linear phase tended to be longer in TA than in GC and SL. The initial rate of decrease in oxy(Hb+Mb) for GC, TA and SL muscle was 12.71 ± 1.17, 15.75 ± 1.19 and 16.54 ± 1.70 %・min^{-1}, respectively. Previous studies have reported similar rates of oxy(Hb+Mb) decline have been reported during resting ischemia, i.e., 21.0 - 23.3 %・ min^{-1} in finger flexor muscles[10] and 19.2 ± 1.2 %・min^{-1} in the medial gastrocnemius muscle[21].

Resting ATP turnover rate. Muscle oxygen store, which was estimated by myoglobin content and capillary supply, was 0.239 ± 0.008, 0.264 ± 0.004 and 0.294 ± 0.008 mmol・(kg wet wt)$^{-1}$ for GC, TA and SL, respectively. Hamaoka et al.[10] suggested that muscle oxygen store at rest was 0.30 mmol・(kg wet wt)$^{-1}$ estimated from NIRS and ^{31}P-MRS data. They[10] also obtained 0.34 - 0.36 mmol・(kg wet wt)$^{-1}$ from previously reported data of oxygenated myoglobin and hemoglobin. As noted, the initial deoxygenation rates were converted to resting ATP turnover rate with correction for muscle temperature decrease (Table 2). There were no significant differences between the muscles. However, there was a positive correlation between % type I fibers and resting ATP turnover rates ($r = 0.748$, $p < 0.01$) as presented in Fig. 2 when the data from all three muscles were pooled.

Discussion

The major finding of this study is the significant positive correlation between % type I fibers and muscle ATP turnover rate at rest (Fig. 2). Little attention has so far been paid to such a relationship. Our results underscore that muscle fiber composition is likely to be one of the factors influencing ATP turnover rate of human muscle at rest. However, no significant difference was observed in the average value of resting ATP turnover in each muscle (Table 2). This may be caused by the fact that oxidative enzyme activity of TA was lower than GC and SL, since the activity of enzymes was better indicator of the functional diversity among locomotor muscles than fiber type composition in human[22].

Previous studies have shown that the PCr decline started later on during resting ischemia compared with oxy(Hb+Mb)[10,21]. This delay was reported to be 4 - 6 min. Our unpublished observation also showed that PCr began to decrease 3.5 - 5.5 min after start of ischemia. Therefore, it can be concluded that anaerobic contribution to resting ATP turnover begins at around 3 - 6 min after start of ischemia. It is possible to detect aerobic contribution during only this time interval. The anaerobic contribution is nearly zero in present study, since the first one min of decline in oxy(Hb+Mb) during ischemia was used for estimating resting ATP turnover rate.

PCr decline rate, which reflects resting ATP turnover rate during ischemia, was measured by ^{31}P-MRS, as noted. The reported values[10,11,21] ranged from 0.40 to 0.60 mmol (kg wet wt)$^{-1}$・kg^{-1} and are somewhat higher than our data (Table 2). Previous studies assumed an ATP concentration at rest of 8.2 mmol (kg wet wt)$^{-1}$ when using for PCr quantification. The

Table 2. The percentage of type I fibers and resting ATP turnover rate estimated by NIRS in gastrocnemius (GC), tibialis anterior (TA) and soleus (SL) muscles.

Muscle	Type I fibres (%)	Resting ATP turnover rate mmol・(kg wet wt)$^{-1}$・min^{-1}
GC	54.0 (5.3)	0.281 (0.046)
TA	62.2 (2.6) ++	0.277 (0.022)
SL	87.7 (3.3) *++	0.366 (0.046)

ATP turnover rate was corrected by muscle temperature decrease. Values are means (SEM) .*($p<0.05$) and ++ ($p<0.01$) denote significant difference from TA and GC, respectively. No significant difference was found in resting ATP turnover rate.

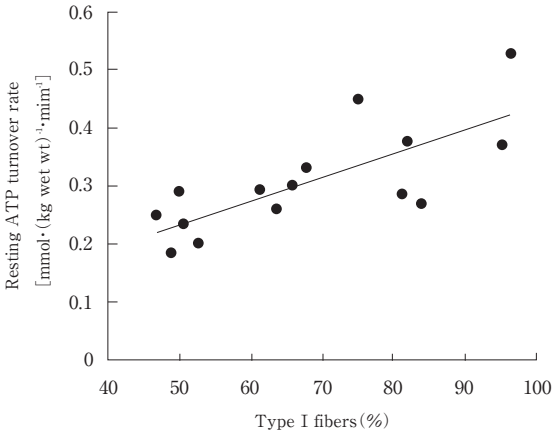

Fig. 2 Relationship between % type I fibers and resting ATP turnover rates. The regression equation describing the relationship is $y = 0.004x + 0.031$, $r = 0.748$ ($p < 0.01$).

correct value when expressed per kg wet weight is about 5 - 6 mmol[23,24]. They also did not take into account of decrease in muscle temperature during ischemia. Some methodological errors seem to contribute the difference among these values.

In rodent muscles, Sullivan and Pittman[9] found that a muscle comprising primarily of oxidative fibers (SO and FOG) showed higher oxygen consumption rate *in vitro* at rest than a muscle in which glycolytic fibers (FG) prevail. Similar observations have been reported for *in vivo* rodent muscles, which oxygen consumption was calculated using resting blood flow and artrioveous oxygen difference[6,7,8]. Sullivan and Pittman[9] also suggested that individual muscle fibers of a mixed skeletal muscle might have different rates of oxygen consumption. Little is, however, known about human resting oxygen consumption.

It is well known that type I fibers in human muscles contains higher number of mitochondria and capillaries surrounding them than type II fibers. In electron micrographic studies[24,25], both total mitochondrial volume and mitochondria fraction expressed as number of mitochondria per unit fiber area (%) showed higher values in type I fibers than type II fibers. Moreover, a 35 % higher activity level of citrate synthase in type I fibers was reported[27]. Similarly, for succinate dehydrogenase a 55 %[28] and a 44 %[29] higher levels in type I fibers was observed. In general, type I fibers have more abundant capillary supply compared with type II fibers. Likewise a number of studies have reported higher capillary to fiber ratio in type I than type II fibers[28,29,30,31].

Type I fibers in human muscles contains higher number of mitochondria and capillaries surrounding them compared with type II fibers. Therefore, type I fibers are superior in oxidative metabolism than that of type II fibers. The present result that resting ATP turnover is closely correlated with % type I fibers of human skeletal muscle is agreement with previous suggestion[6,7,8,9]. Fiber type composition is potentially an important factor in setting the resting metabolic rate in humans *in vivo*.

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筋繊維組成は近赤外線分光法により評価されたヒト虚血筋における安静時ATP代謝量に影響を与える

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要　旨

本研究の目的は近赤外線分光法を用いて、虚血された腓腹筋、前脛骨筋およびヒラメ筋における安静時のATP代謝速度と筋線維組成の関係を検討することである。腓腹筋、前脛骨筋およびヒラメ筋の遅筋線維占有率はそれぞれ54.0 ± 5.3、62.2 ± 2.6（$p < 0.05$）および87.7 ± 3.3%（$p < 0.01, p < 0.01$）であった。近赤外線分光法によって測定された虚血初期の筋酸素化レベルの低下率は、安静時における筋内酸素貯蔵量とリン酸／酸素比（5.5）を用いて、安静時ATP代謝速度に変換された。また、安静時ATP代謝速度を、虚血時における筋温の低下を考慮して補正した。腓腹筋、前脛骨筋およびヒラメ筋における安静時ATP代謝速度はそれぞれ、0.281 ± 0.046、0.277 ± 0.022および0.366 ± 0.046 mmol (kg wet wt)$^{-1}$·min^{-1}であった。各筋の値をまとめると、安静時のATP代謝速度は遅筋線維占有率と有意な正の相関関係（$r = 0.748, p < 0.01$）にあった。以上の結果から、遅筋線維占有率は*in vivo*の測定においても、安静時代謝量を決定する重要な要因であると示唆される。

＜キーワード＞近赤外線分光法、%タイプⅠ線維、安静時代謝、前脛骨筋