Heterogeneity of Fiber Types in Human Observed ^{31}P NMR Spectroscopy during Exercise.

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Abstract

Phosphours nuclear magnetic resonance (^{31}P NMR) spectroscopy was used to evaluate the relationship between changes in the concentrations of muscular phosphate metabolites during exercise and recovery and muscle fiber composition of M. Vastus lateralis in human. The subjects were divided in the three fiber type groups (Slow group ; $<$ 40 % FT fibers, n = 8, Intermediate group ; from 40 to 60 % FT fibers, n = 16, Fast group ; $>$ 60 % FT fibers, n = 6). The fast group showed a significantly lower PCr / Pi ratio than those of the intermediate and slow group at the first minute of exercise. This tendency was also observed after the first minute of exercise and during recovery. The slow group showed the highest recovery rate in the PCr / Pi ratio during the first minute after the exercise. On the other hand, the fast group showed the slowest recovery. It is suggested that PCr / Pi ratio during exercise and recovery by ^{31}P NMR relates to the differences in muscle fiber types.

^{31}P NMR, muscle energetics, phosphocreatine, exercise, muscle fiber types

Introduction

Muscle is composed of two types of fiber with opposite characters, i,e., fast-twitch (FT) at slowtwitch (ST) fibers. The ratio of these two types (muscle fiber composition) is closely related to aptitude of sport events[3,13]. The biopsy had been only one modality to determine muscle fiber composition in human. The development of a noninvasive approach could be valuable to the field of sport science and medicine.

Nuclear magnetic resonace (NMR) noninvasively provides information on metabolites which has not been obtained in vivo by conventional methods. Recently, there are some reports on the use of NMR[2,6,8,15]. Using magnetic resonance imaging (1H-MRI), we have found an extremely high correlation between magnetic resonance relaxation time (T1) and muscle fiber composition by biopsy[4,5]. On the other hand, we indicated no definite relationship between phosphocreatine (PCr) / inorganic phosphate (Pi) by ^{31}P NMR spectroscopy at rest and % FT fibers[7]. Some gruops[1,12] examined that the relationship between fiber types and the split peaks of Pi observed with ^{31}P NMR spectra during exercise. They suggested that it might be possible to characterize fiber types. However, in these studies, whether the ^{31}P NMR results actually reflected muscle fiber compostition it was not confirmed by muscle biopsy.

In the present study, using ^{31}P NMR during exercise and recovery, we investigated the relationship between phosphorus metabolites of human muscle and fiber types in the subjects whose muscle fiber composition had been determined by biopsy.

Methods

Subjects

The subjects in this study wer 30 male of students (specialized physical education), ages 20-26 years, ranging in height from 165.2～180. 5cm (mean=171.7cm, SD=4.6) and in weight from 60.4 to 75.5kg (mean=68.2kg, SD=4.9). Informed consent to partici-pate in the study was obtained from all subjects.

Muscle type.

The needle biopsy specimens (about 20mg) were taken from the quadriceps fenoris muscle (musclus vastus lateralis) by an orthopedic surgeon. The biopsy site was 35～40% of the distance from the superior margin of patella to the anterior superior illac spine. The samples were immediately frozen in lsopentance and cooled by liquid nitrogen. From histochemical analysis, sequential 10-μm sections were cut in a cryostat maintained at −20℃, and stained for myofibrillar ATPase activity. Based on the ATPase staining after pre-incubation (pH 10.3), fibers were classified into two main types, FT and ST. We counted the 400～600 fibers for each subject to decide the muscle fiber composition.

According to the muscle fiber composition of M. vastus lateralis determined, the subjects were classified into 3 groups : fast group (predominantly FT fibers, >60% FT fibers), intermediate group. (from 40 to 60% FT fibers), slow group (predominantly ST fibers, <40% FT fibers).

^{31}P NMR.

^{31}P NMR spectoroscopy were done more than 1 month after muscle biopsy. All subjects refrained from exercise for 24hours before the study. A spectrometer (Signa; GE Medical Systems, Milwaukee, U.S.A.) was used with a superconducting magnet at 1.5 Tesla with 60cm bore[7]. ^{31}P NMR measurements were performed at 25.9MHz with a 3.5inch diameter (transmit) surface coil placed on the right M. vastus lateralis. That position was fixed to allow biopsy site at the coil center. NMR spectra were collected by accumulating 6 transients with a recycling time of 3.5 sec. Quantification

of metabolites was carried out by integrating NMR peak areas. To avoid the partial saturation factors obtained by a comparison of peak area was calibrated using the saturation factors obtained by a comparison of peak areas measured with recycling times of 3.5 sec and 15 sec. Changes in PCr and Pi were evaluated as the PCr/Pi ratio. Measurement of NMR was carried out continuously from the resting period to the exercise period (4 min) and the recovery period (5 min). The subjects lay in the supine position with a weight about 1 kg fixed on the right ankle and lifted the ankel with the knee extended at a rate of 60 times per minute for 4 minutes. Care was taken to make the distance of each lifting uniform.

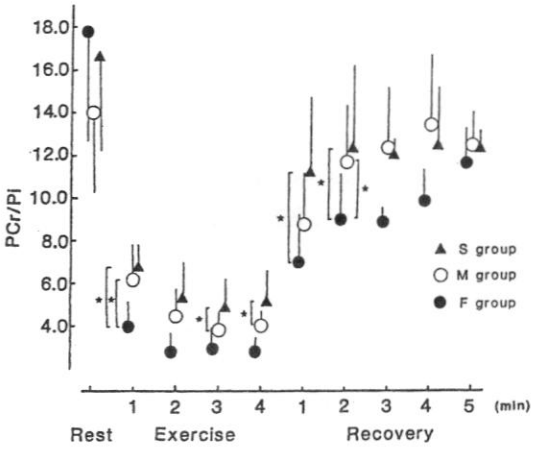

Fig.1 Changes of PCr/Pi during exercise (4 min)and recovery (5 min) on the different fiber type groups. Values are means±SD. for one minute, ▲ Slow group (29.1±5.1 %FT fibers;n=8), ○ Intermediata group (46.8±5.1%FT fibers;n=16), ● Fast group (71.9±11.1 %FT fibers ; n=6), : $p < 0.05$

Results

The determined fast, intermediate and slow groups by muscle biopsy consisted of six subjects (mean±SD values=74.9±11.1% FT fibers), sixteen subjects (46.8±5.1% FT fibers) and eight subjects (29.1±5.1% FT fibers), respectively. The % FT fibers of Fast gruop has a significant difference ($p<0.05$) from intermediate and slow groups and the one of intermediate group also in higher than slow group ($p<0.05$).

Figure 1 shows changes in the PCr/Pi ratio in the three groups before, during and after exercise. During the first minute of exercise, the fast group showed a significantly lower PCr/Pi ratio than did the other two groups ($p<0.05$). This tendency was also observed after the first minute of exercise and during recovery. The slow group showed the highest recovery rate during the first minute after discontinuation of exercise, and the fast group showed the slowest recovery.

Discussion

Several research groups[1,12] have reported differences in metabolism between the FT and ST fibers by NMR. Using ^{31}P NMR, Chance's group[12] found that inorganic phosphate (Pi) shows two peaks during exercise and suggested that these peaks indicated different intracellular environments of the FT and ST fibers. These findings were also supported by Achten et al[1] . On the other hand, we focused on the relaxation time calculated from 1H-magnetic resonance imaging (MRI) and found that the relaxation time at rest was related to the muscle fiber composition[4,5]. However, the relationship between muscular metabolism during exercise and muscle fiber composition by biopsy has not been investigated so far.

In this study, the fast group showed a significantly lower PCr/Pi ratio than those of the intermediate and slow groups at the first minute of exercise. This tendency was also observed during the subsequent period of exercise and recovery. FT fibers have low aerobic capacity and use glycolysis as a main

ATP source and produce many protons[14]. It is established that FT fibers have higher creatine kinase activity and, hance, higher Pi concentrations during exercise, and that the recovery is slower than ST fibers[13]. It is reasonable, therefore, that the ratio of PCr/Pi of fast group is lowest during exercise as was shown in this study.

The ATP demand decreases, immediately after the exercise discontinues, ATP is mainly used for phosphorylation of creatine to PCr. Since ATP is produced by oxidation after exercise, recovery of the PCr level reflects the oxidative faculty during exercise[2,15,16]. In particular, recovery of the PCr level in the inital stage of recovery is attributable to the resynthesis of ATP in mitochondria[16]. The higher PCr/Pi of the slow group than that of the fast group at the first minute of recovery suggests that the slow group preserved a higher faculty for mitochondrial respiration. This is highly likely in view of the higher oxidative faculty in the slow group than that in the fast group.

In conclusion, determination of the PCr/Pi ratio classified according to the muscle fiber composition during exercise and recovery revealed that the ratio in the fast group was lowest in both periods, reflecting differences in the metabolic properties of FT and ST fibers. Since ^{31}P NMR used in this study permits noninvasive measurement of phosphorus metabolites and quantification of differences in fiber composition, it is suggested that the ratio of PCr/Pi by ^{31}P NMR can be used to predict the three fiber type groups.

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