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T. Kanayama[1*], K. Minowa[2], N. Inoue[4], T. Yamaguchi[4], T. Tamura, S. Yoshida[3] and T. Kawasaki[1]

[1]Department of Prosthetic Dentistry I, [2]Dental Radiology, and [3]Oral Anatomy I, School of Dentistry, Hokkaido University, N13 W7, Kita-ku, Sapporo 060-8586, Japan

[4]Special Clinic for Specific Disorders, Dental Hospital, Hokkaido University, N13 W6, Kita-ku, Sapporo 060-8586, Japan; *corresponding author

T. Kanayama: Department of Prosthetic Dentistry I, School of Dentistry, Hokkaido University, N13 W7, Kita-ku, Sapporo 060-8586, Japan

E-mail: kanayama@den.hokudai.ac.jp

Tel. +81-11-706-4221

Fax. +81-11-706-4928

Short title: **Metabolism of Masseter and Medial Pterygoid muscles**

Key words: masseter, medial pterygoid, spectroscopy (^{31}P-MRS), chemical shift imaging (CSI), metabolism.

Summary. The aim of this study is to compare phosphocreatine (PCr) concentrations of human masseter and medial pterygoid muscles by a recently developed localized MRS method, Chemical Shift Imaging (CSI). The characteristic spectra of phosphorus metabolites including PCr and β-ATP from the superficial part of the masseter (SM) and the deep part of the masseter (DM) and the medial pterygoid muscles (MPt) from 11 volunteers, 20-27 years-old were obtained. The study clearly demonstrated higher PCr/β-ATP in the SM and MPt than in the DM both in mean values ($p<0.01$) and in individual subjects. The results indicate that SM and MPt are power producers. There were no significant differences in the mean values of the PCr/β-ATP ratios in SM and MPt, however, the PCr/β-ATP ratios varied individually and the subjects could be divided into three distinct groups: values of MPt higher than SM (group A, 4 subjects); values of MPt almost equal to SM (group B, 3 subjects); and values of MPt lower than SM (type C, 4 subjects). There appears to be a close relationship between the PCr content as determined in the groups here and occlusal guidance.

Introduction

Bioptical methods are conventionally used to measure the concentrations of so-called high-energy phosphates in muscles, however, these methods require tissue samples from the subjects. Recently, ^{31}P-Magnetic Resonance Spectroscopy (^{31}P-MRS) has been widely applied to the study of the muscular metabolism (Meyer *et al.*, 1982; Boicelli *et al.*, 1989; Bernus *et al.*, 1993), because it provides noninvasive measurements of phosphocreatinine (PCr) concentrations in muscles. The PCr is an energy-rich phosphate in muscles, and its concentration in the muscle is considered to relate to the muscle contractile force (Park *et al.*, 1988; Vandenborne *et al.*, 1995).

Formerly, ^{31}P-MRS studies had been performed with surface-coils and simple pulse-acquire sequences (Taylor *et al.*, 1983; Arnold *et al.*, 1985), however the lack of sensitivity and signal localization limits examination to only the superficial site. To overcome this limitation, a new localized MRS method, Chemical Shift Imaging (CSI), has been developed to examine deeper parts of the muscle. This new method has succeeded to obtain clear signals from human masseter muscle, and has allowed measurements of the different contents of PCr in the deep (DM) and superficial parts (SM) of the masseter (Kanayama *et al.*, 2000).

The medial pterygoid muscle (MPt) is known as a deep counterpart to masseter, and it acts to elevate the mandible with the masseter. However the MPt has not been studied with MRS because of the inaccessibility of the muscle. The present study examined the PCr contents of the medial pterygoid muscle, and compared the results with the deep and superficial part of the masseter.

Materials and Methods

Subjects

Eleven male volunteers aged 20-29 years-old participated in the study. Local ethics committee approval and informed consent are obtained for each subject. All subjects had full dentition, and were free from orofacial pain or pathologies of the masticatory system.

Magnetic Resonance examination

Magnetic Resonance experiments were performed on a Siemens Magnetom Vision whole-body 1.5T MRI/MRS system with a 14 cm diameter, dual-tuned, 64/25 MHz $^{1}H/^{31}P$, radio frequency surface-coil. First, the coil was shimmed using the proton frequency until the water peak dropped down to 0.5 ppm full width half maximum (FWHM), then survey serial Magnetic Resonance images from orbita to mandible were obtained to verify the position of VOI over each part of the Mas and MPt. The DM and SM were distinguished by the existence of aponeurosis between the two (Minowa *et al.*, 1998). The slice selection was made by slice selective gradient in the long axis direction for masseter and in the sagittal axis direction for medial pterygoid, and 2D-CSI was performed with each of 16 phases encoding in two dimensions. Slice thickness was 30 mm and the focus of view (FOV) was 320 mm. The voxel dimensions after k-space zero filling were 1x2x3 cm^3.

Figure 1 shows the cross-sectional MR images of DM, SM, and MPt. All measurements were performed on almost the same portion as Fig. 1 in each subject. Raw data were acquired with a repetition time of 330 ms by 9 transients, resulting in a measurement duration of 12 min and 30 sec. Before Fourier transformation the sum of the free

induction decays (FIDs) were multiplied by an exponential with decay chosen to correspond to a 6.4 Hz line broadening. After the Fourier transformation, all spectra were adjusted with zero- and first-order phase corrections. The integrals of the resonance areas from the PCr and β-ATP peaks, which represent the amounts of phosphate in vivo MRS, were measured by a fitting procedure using lorentzian line shapes.

This study evaluated the PCr level in the muscle with the use of PCr/β-ATP ratios. Because (1) traditionally, human ^{31}P-spectra are quantified in relative terms only, (2) PCr/ATP is considered as an index of the energetic state (Neubauer *et al.*, 1998), and (3) the peak of β-ATP was higher in all ATP, and most stable in the spectra (Marcel, 1995; Kanayama et al., 2000). The data were subjected to a Sheffe's F test with the level of significance set at $P<0.05$.

Prior to the start of the examination, repeated measurements on a single individual were carried out 6 times over 6 weeks to determine the reproducibility of the CSI results on each muscle (Fig. 2), then the CSI examinations were performed with the 11 subjects. The relative SE [(SE/mean)x100] of the PCr/β-ATP in the SM, DM, and MPt for the 6 measurements were 0.8, 1.3, and 1.1% respectively.

Result

Fig. 3 shows typical phosphorus MR spectra obtained from each muscle at rest. Several resonances reflecting the high-energy phosphate concentrations, such as β-ATP, PCr, and Pi could be detected. The phosphomonoester (PME) and phosphodiester (PDE) peaks, which reflect sugar-phosphates and membrane phospholipids metabolites, could also be observed. Although it is difficult to establish the absolute phosphate content by

these data alone, the peak and integral values of PCr in the DM were lower than in SM and MPt. The values of β-ATP were very similar in all three.

The mean values of PCr/β-ATP were 4.68 in the SM, 3.51 in the DM, and 4.65 in the MPt (Table). The PCr/β-ATP ratio was significantly ($p<0.01$) higher in the SM and MPt than in the DM, while no significant differences were found between SM and MPt.

Fig. 4 shows the integral values of PCr/β-ATP in the three muscles for all the 11subjects. Similar to the results of the mean values described above, the values of PCr/β-ATP ratios in all subjects were higher in the SM and MPt than in the DM except for one subject. However, the values of the PCr/β-ATP ratios between the SM and MPt varied individually. The subjects were divided into three distinct groups: values of MPt higher than SM (group A, 4 subjects); values of MPt almost equal to SM (group B, 3 subjects); and values of MPt lower than SM (type C, 4 subjects).

Discussion

A recently developed localized MRS method, Chemical Shift Imaging (CSI), enabled obtaining typical MR spectra of the superficial (SM), and deep part of masseter (DM), and medial pterygoid muscle (MPt) from all subjects examined. So far it has been impossible to examine details of the metabolism of deep muscle, and this is the first report applying this method to the human MPt.

Prior to the study, the PCr/β-ATP ratio of the SM, DM, and MPt of one subject were repeatedly (6 times) examined over 6 weeks to determine the reproducibility of the CSI results of each muscle. The relative SE [(SE/mean)x100] was 0.8% (SM), 1.3% (DM), and 1.1% (MPt) (Fig. 2). The variability between these measurements is a

combination of experimental errors and physiological variations in the subject. The result amply demonstrated the reproducibility of the measurements.

The study clearly demonstrated higher contents of PCr in the SM and MPt than in the DM both in mean values and in individual subjects. The results indicate that the SM and MPt are power producers. This is consistent with histochemical evidence that the posterior parts of the SM and MPt are characterized by a relatively high frequency (about 45%) of large diameter type IIB fibers. Type IIB fibers belong to fast-twitching, rapidly-contracting motor units, which generate large forces and are best suited for bursts of intense, intermittent activity (Eriksson and Thornell, 1983). In contrast, DM is characterized by relatively high frequency of large type I fiber (anterior portion 72% and posterior portion 62%). The muscle fibers of DM arranged vertically with a high concentration of complex spindles in a direction favorable to sense changes in stretch. Therefore DM is considered to be well adapted for mandibular postural control and locomotion (Eriksson and Thornell, 1983; Eriksson and Thornell, 1987).

There were no significant difference in the mean values of PCr/β-ATP ratios of SM and MPt. This result is supported by MPt being a deep counterpart of masseter that acts as an elevator of the mandible with masseter, and as many similarities in these two muscles have been reported by EMG (Gibbs *et al.*, 1984) and histochemically (Eriksson and Thornell, 1983). The study here shows a metabolic resemblance between SM and MPt.

Different from the results of the mean values, the integral values of the PCr/β-ATP ratios between SM and MPt varied individually. Comparing the relative amounts of PCr in these two muscles, the subjects could be divided into three distinct groups: values of MPt higher than SM (group A, 4 subjects); values of MPt almost equal to SM

(group B, 3 subjects); and values of MPt lower than SM (type C, 4 subjects).

Some studies have suggested that occlusal guidance influences the EMG activity of the elevator muscles, and that group function was associated with higher elevator EMG activity (Belser and Hannam, 1985; Watanabe *et al.*, 1998; Kahn *et al.*, 1999), and canine guidance with lower EMG activity when the subject performs lateral movements. So we checked the occlusal conditions of all subjects as one of the factors which may influence the muscular metabolism. As a result, all group A subjects save one had less occlusal wear and canine guidance during lateral excursion of the mandible. The group B subjects all had the group function. Group C subjects all displayed pronounced occlusal wear and thus provided a distinct group function on the working side, and all of these subjects had molar balancing contact on the supporting side.

Since this study only examined the occlusal guidance (and not occlusal contact patterns or occlusal forces) of the subjects, it is not possible to evaluate details of the muscle loads with these data only. It was also not possible to determine the statistical differences between the groups. However, the results suggest that the eccentric occlusal conditions affect PCr amounts in the MPt, and there seems to be two possible explanations for the differences: 1) many tooth contacts in the eccentric position produce a stable platform to supply force leading to PCr in the MPt to produce large force at that position; or 2) PCr-rich MPt at the eccentric position causes tooth wear, and the occlusal contact increases as a result. Further detailed analysis must be made with subjects classified by occlusal condition and a statistical between group analysis is necessary to elucidate the reasons for the differences.

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Table 1

Relative concentration of PCr in the superficial part of masseter (SM), deep part of masseter (DM), and medial pterygoid (MPt) muscles (Mean±SD).

	SM	DM	MPt
PCr/β-ATP	4.68*±0.60	3.51±0.49	4.65*±0.75

* Significantly higher compared with deep part of masseter ($p<0.01$ by Sheffe's F test).

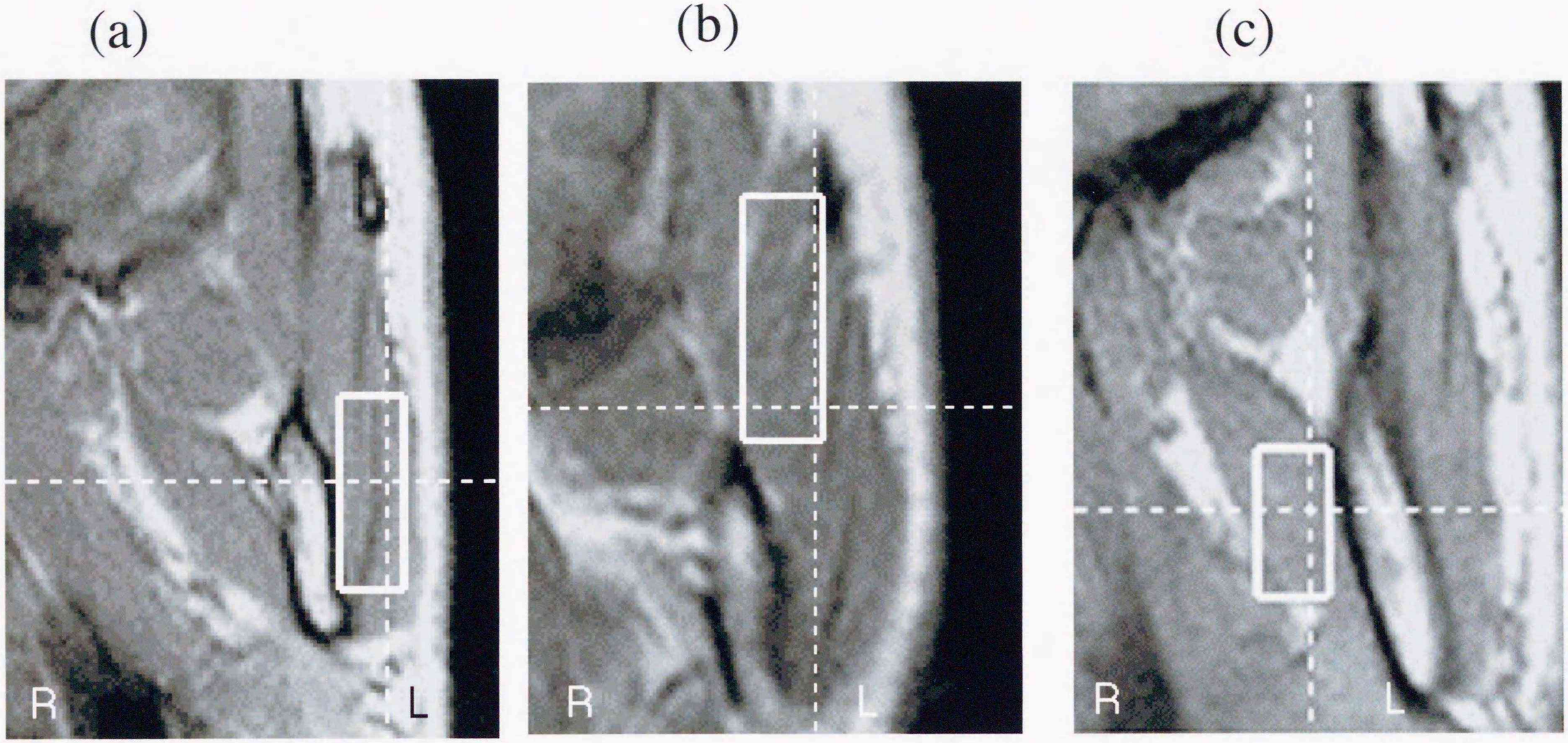


Figure 1
The T1-weighted coronal images of the left superficial part of masseter (a), deep part of masseter (b) and medial pterygoid muscle (c) with the volume of interest ($1\times2\times3\times cm^3$).

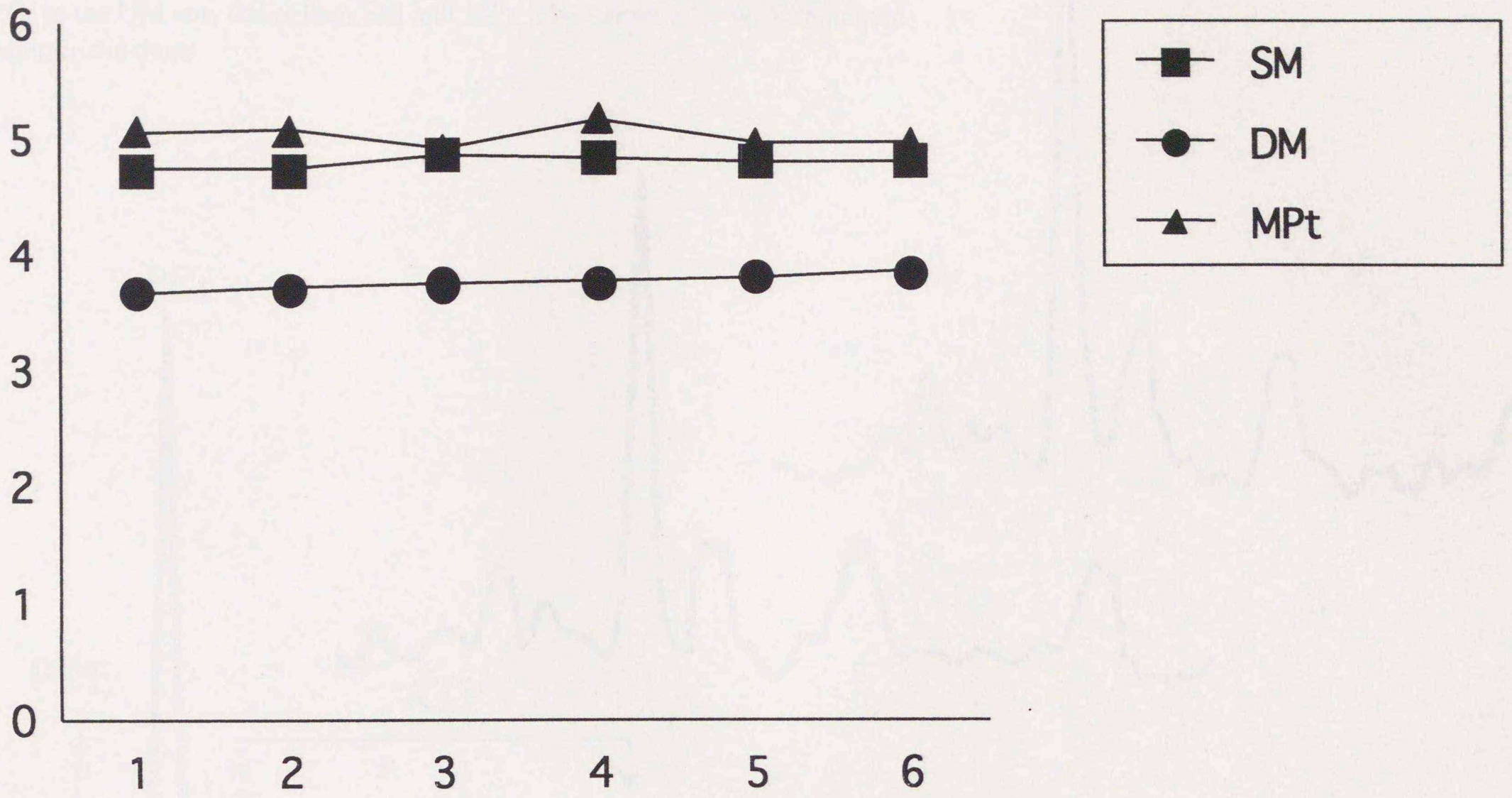
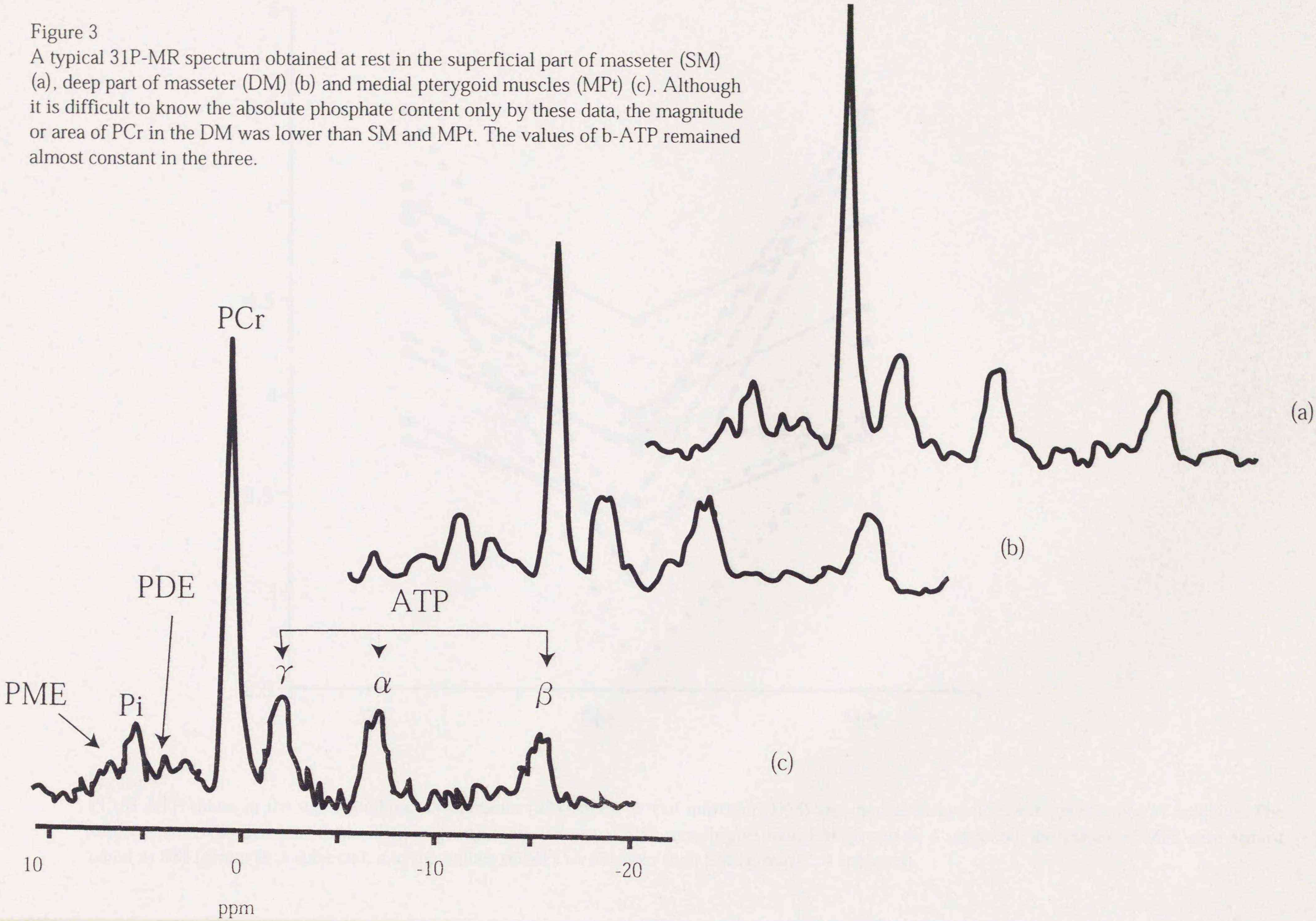


Figure 2
The relative SE [(SE/mean)×100] for the localized measurements in the superficial part of masseter (SM), deep part of masseter (DM) and medial pterygoid muscle (MPt) was 0.8, 1.3, and 1.1% for the PCr/β-ATP ratio, respectively.

Figure 3
A typical 31P-MR spectrum obtained at rest in the superficial part of masseter (SM) (a), deep part of masseter (DM) (b) and medial pterygoid muscles (MPt) (c). Although it is difficult to know the absolute phosphate content only by these data, the magnitude or area of PCr in the DM was lower than SM and MPt. The values of b-ATP remained almost constant in the three.



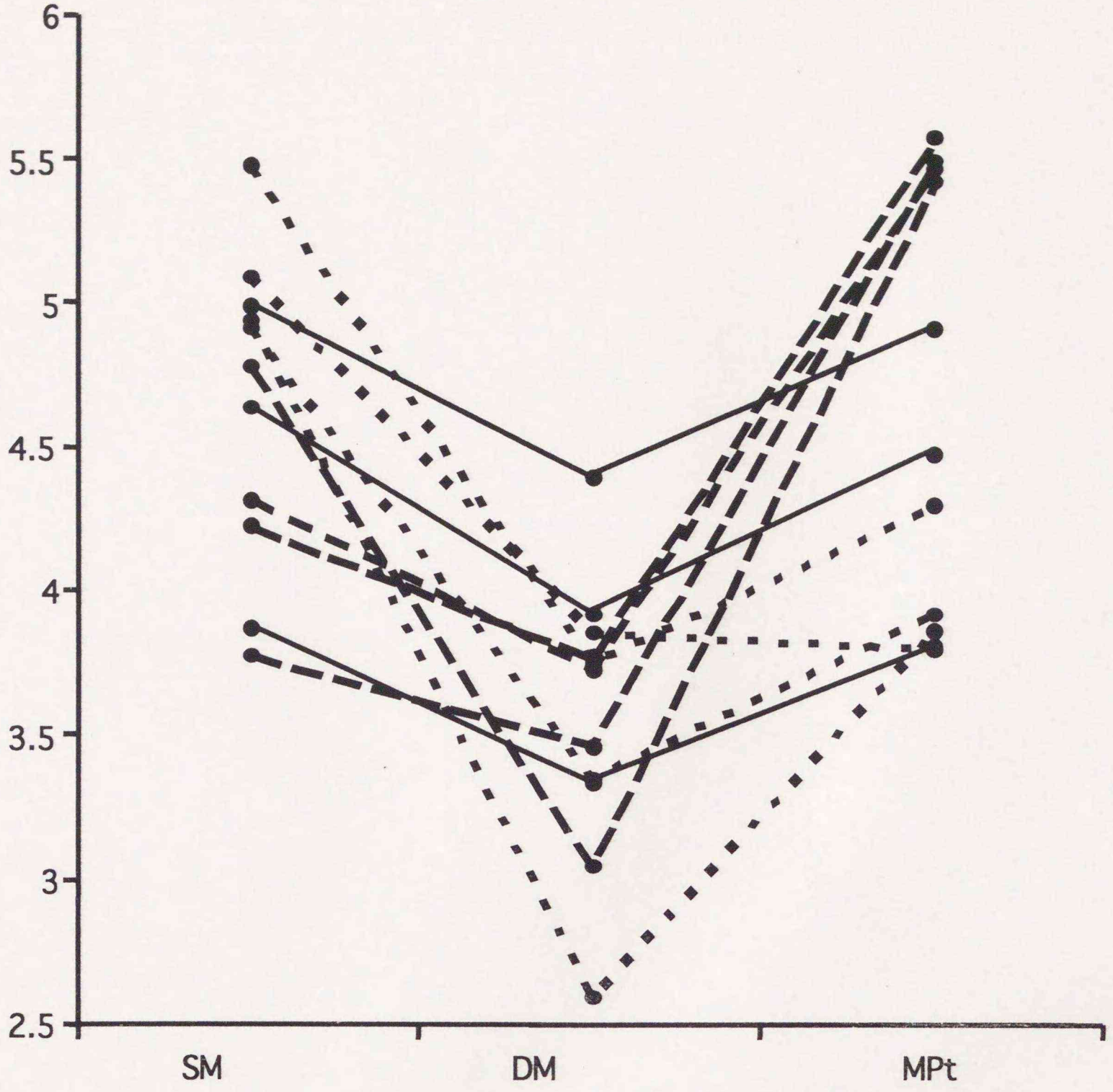


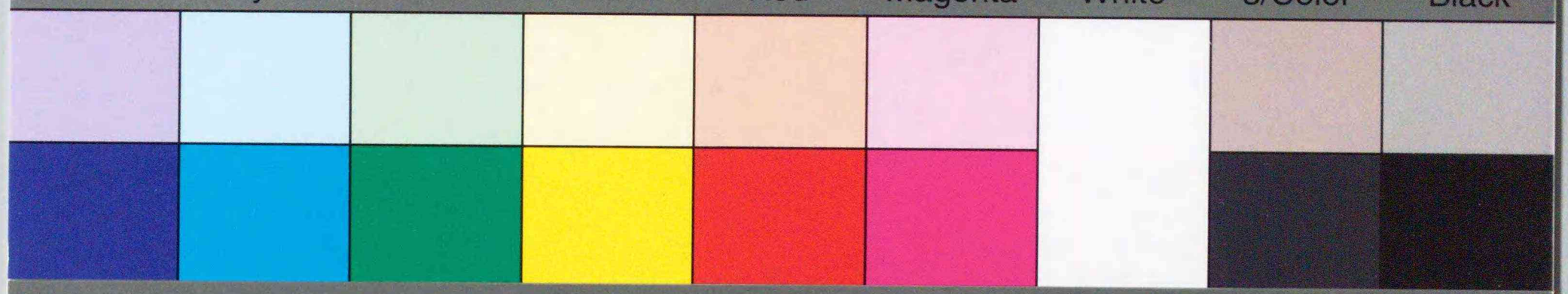
Figure 4
PCr/β-ATP ratios in the superficial part of masseter (SM), deep part of masseter (DM) and medial pterygoid (MPt) muscles of 11 subjects. The subjects were divided into three distinct groups: the values of MPt were higher than SM (group A, 4 subjects); the values of MPt were almost equal as SM (group B, 3 subjects); and the values of MPt were lower than SM (group C, 4 subjects)

inches 1 2 3 4 5 6 7 8

cm 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

Kodak Color Control Patches

Blue Cyan Green Yellow Red Magenta White 3/Color Black



Kodak Gray Scale

C Y M

A 1 2 3 4 5 6 **M** 8 9 10 11 12 13 14 15 **B** 17 18 19