Fiber types and diameters in the porcine masseter muscle

A histochemical study

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Type I, I:B, II:A, and II:C fibers were identified by adenosine triphosphatase histochemistry in masseter muscles from 22 female pigs (1 year old, 70–90 kg body weight). Type II:B fibers were not found. This was in contrast to the findings of five fiber types in the porcine soleus muscles. In the porcine masseter the most prominent fiber type was II:A (75%). Type I fibers constituted 15% of the fiber types on average. Type I:B and II:C fibers were less frequent (4–6%). No significant difference was found between various biopsy locations, but there was a tendency towards more type I fibers in the deeper part of the masseter muscle. The mean fiber diameters were larger in the masseter muscles than in the soleus; however, the differences were significant only for fiber type I:B. □ *Histocytochemistry; masseter muscle; myosin adenosine triphosphatase*

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The most widely used histochemical method for classifying mammalian muscle fibers into distinct fiber types is the myofibrillar adenosine triphosphatase (ATPase) technique (1, 2). This procedure enables, after acid and alkalic preincubation followed by a routine ATPase incubation, separation of the fiber population into major fiber types (type I and type II) and subtypes (type II:A and type II:B) (3–6). Furthermore, transitional fiber types such as type II:C fibers, showing intermediate staining modalities between these major fiber types, can be detected (7, 8). The validity of the fiber type concept can be increased by combining ATPase and metabolic-based systems. The relationships between these classification procedures and physiologic variables such as contraction speed (9, 10) and fatiguability (4, 11, 12) enable the fiber type composition to provide additional information about muscle function (13).

The histochemical demonstration of ATPase activity is sensitive to many factors such as time, temperature, acidity and the composition of the preincubation medium (14, 15). This technique must therefore be applied in a standardized fashion, using of reference muscles and strictly controlled conditions to make the results comparable and repeatable (16, 17). This is especially important when muscle groups such as jaw muscles are studied. The masticatory muscles diverge in fiber type composition, distribution, and size in comparison with limb and trunk muscles (18–22). This is reflected in a complex internal architecture and a highly specialized function (23–26).

Gross anatomic studies have shown that the porcine craniomandibular joint (CMJ) and masticatory muscles resemble the human masticatory system (27, 28), and the porcine masseter muscle has been used in physiologic studies (29, 30). However, very few attempts have been made to study the fiber type composition of the porcine masseter muscle. Schiaffino (31), Suzuki (32), and Suzuki & Cassens (33, 34) evaluated the fiber type profile of the pig masseter muscle, but biopsy specimens were taken only from

the superficial and central parts of the masseter muscle. Furthermore, a classification of type II fibers into type II:A and II:B subgroups was not performed (32). The presence of type II:A and II:B fibers in the pig masseter muscle is also controversial. Ström (35) found no classical type II:B fibers in this muscle but was able to distinguish the fiber type in the porcine soleus muscle. Tuxen & Kirkeby (36) separated a small fraction (2–5%) of type II:B fibers in the pig masseter. However, these results are contradictory to the findings of Tuxen & Rostrup (37), who stated that the type II:B fibers predominated and the type II:A fibers were present in only 3 of 36 porcine masseter muscle biopsy specimens. Thus the purpose of this study was to examine the histochemical separation of fiber types in the porcine masseter muscle, with special emphasis on the separation and identification of type II:A and type II:B fibers.

Materials and methods

Twenty-two adult Swedish domestic pigs (Sus Scrofa Domesticus, 1 year old, 70–90 kg body weight, all female) were used. The pigs were anesthetized with an intramuscular injection of 15–20 mg/kg body weight ketamine (Ketalar®, 50 mg/ml, Parke-Davis, Morris Plains, N.J., USA) before removing the masseter muscles. After experimentation the animals were killed with an overdose of the anesthetic, followed by an intravenous injection of potassium chloride. This project was approved by the Ethical Committee at the University of Göteborg.

Histochemical analyses

Fiber type classification. Biopsy specimens were obtained from the anterior, central, and posterior aspects of 15 right and 15 left masseter muscles, representing the whole superficial–inferior distance down to the mandibular ramus. Central portions of the soleus muscles from five pigs were included as reference muscles.

Biopsy specimens for histochemical analysis were mounted in an embedding medium (OTC® compound, Histo-Lab, Bethlehem Trading Ltd, Göteborg, Sweden) and frozen in isopenthane (Kebo AB) chilled with liquid nitrogen (−140°C), and stored at a temperature of −80°C for later analysis.

Serial transverse sections were cut in a cryostat (System Dittes-Duspiva, Heidelberg, Germany) at −20°C with a thickness of 10 μm and stained for myofibrillar ATPase (3). The individual muscle fibers were identified after alkalic (pH 10.3) and acid preincubation (pH 4.5, 4.4, and 4.3) as type I, I:B, II:A, II:B, and II:C (5, 6, 14, 15, 38). The oxidative capacity was evaluated by nicotinamide adenine dinucleotide, reduced form (NADH)-tetrazolium oxidoreductase, a mitochondrial-bound oxidative enzyme (39) modified by Nyström (40). Photographs were taken with a Zeiss microscope using Agfapan 25 professional film.

Fiber type composition and fiber sizes. In six left porcine masseter muscles biopsy specimens were obtained from the anterior, central, and posterior parts of the intermediate portion (35). The samples were all taken 15 mm from the surface, with the same surface–ramus distance (Fig. 1). Furthermore, in four other left pig masseter muscles the antero–posterior distance was constant, and fibers originating from the central muscle representing the whole superficial–inferior distance down to the ramus of the mandible were collected. No muscle sample contained less than 200 fibers. Fiber sizes were estimated in four masseter muscles and four soleus muscles by the method of minimum (least) diameter (5, 41). A minimum of 30 fibers of each fiber type in all counted sections was included in the calculation of mean least diameter.

Statistical analyses

The variability of fiber type proportions and fiber diameters was expressed by the standard deviation (SD). Analysis of variance, Student's *t* test, and Wilcoxon's signed rank test were used to test the null hypothesis in proportion and diameter between different fiber types and locations.

Results

Histochemical profile

Soleus muscle. The muscle fibers were classified after alkalic preincubation (pH 10.3) into light-stained alkaline-labile type I fibers and dark, alkaline-stable type II fibers. Both type I:B and II:C fibers displayed an intermediate ATPase staining (Fig. 2).

Preincubation at pH 4.5 and 4.4 distinguished weakly stained type II:A fibers. Type II:B fibers stained moderately. The separation of type II:A and type II:B fibers was more prominent at pH 4.4. Type I, I:B, and II:C fibers were dark. At pH 4.3 type II:A and II:B fibers showed an acid reversal pattern with an inhibited ATPase activity. Type I, I:B, and II:C fibers were dark.

NADH histochemistry showed strong activity in type I, I:B, and II:C fibers. Type II:A fibers stained moderately to strongly, whereas most of type II:B fibers stained weakly. About 10% of type II:B fibers displayed a medium intensity of NADH activity (Fig. 2).

Masseter muscle. Preincubation at pH 10.3 identified type I and type II fibers. Furthermore, intermediate type I:B fibers displaying a weak ATPase staining could be detected. Transitional forms of type II:C fibers showing strong to medium ATPase reactivity were present (Fig. 3). At pH 4.5 type I, I:B, and II:C fibers showed a reversed ATPase reaction, staining strongly. Type II fibers had a moderate staining intensity. Preincubation at pH 4.4 and 4.3 did not change the localization of the ATPase product in type I, I:B, and II:C fibers. However, an acid reversal was evident in type II fibers with an inhibited ATPase reaction after preincubation at pH 4.4 and pH 4.3. These acid-labile type II fibers were classified as type II:A. Classical type II:B fibers with a strong to moderate ATPase reaction at pH 4.5 and pH 4.4 as in the porcine soleus muscle could not be identified. In the porcine masseter muscle all fibers showed a medium intense staining for NADH-dehydrogenase. However, a tendency was found in type I, I:B, and II:C fibers for strong NADH activity. It was not possible to distinguish type II:A and type II:B fibers on the basis of oxidative capacity (Fig. 3).

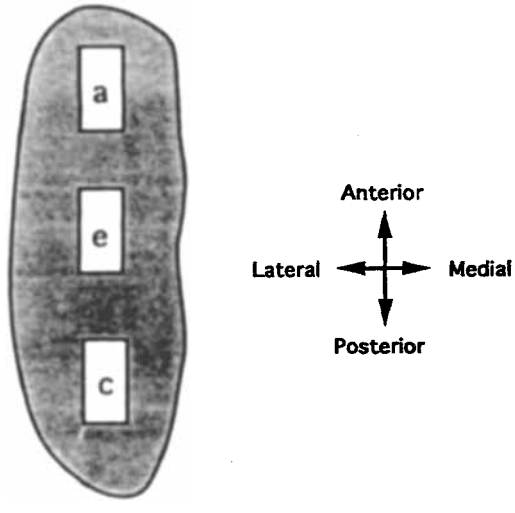

Fig. 1. Schematic illustration of sagitally oriented left porcine masseter muscle. Cross-section from above. a = antero-, e = centro-, and c = postero-intermediate muscle biopsies.

Fiber type frequency

The total number of investigated muscle fibers and the relative frequency of type I and type II fibers from the anterior, central, and posterior parts from the intermediate part of six porcine masseter muscles are shown in Table 1. No classical type II:B fibers could be detected, and type II:A was the dominant fiber type, representing three-quarters of all fibers. Fifteen per cent were type I fibers, whereas type I:B and II:C were less frequent (4–6%).

Even though there was no significant difference between fiber type distribution and biopsy location, there were, however, large variations in interindividual fiber type proportions. This variation was most pronounced for type I fibers, for which the interbiopsy variation ranged from 5% to 28%. The central biopsy specimen displayed the highest frequency of type I fibers (18%) and the lowest population of I:B fibers (5%) in comparison with the anterior and posterior muscle samples. The antero–intermediate specimens showed the highest

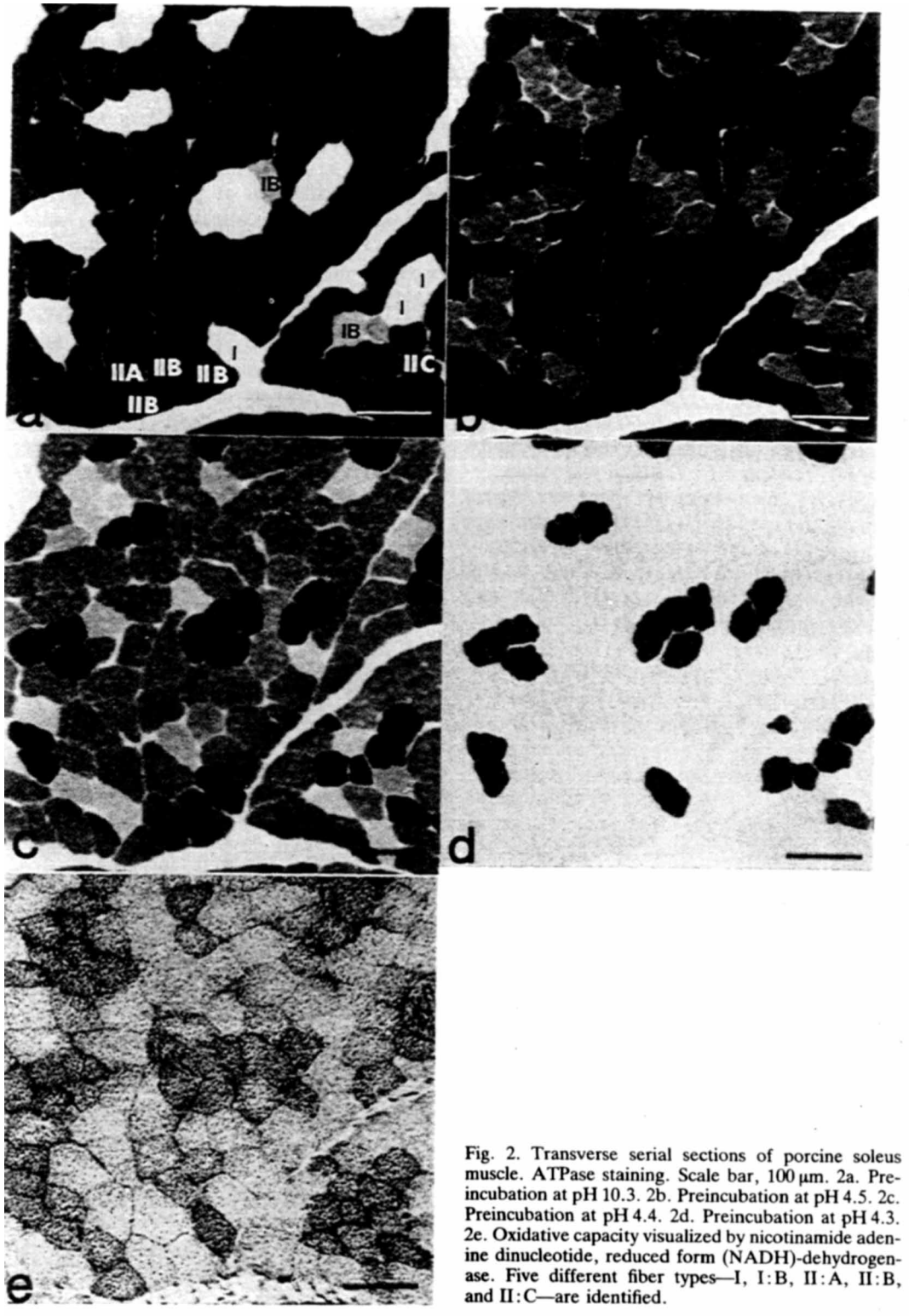

Fig. 2. Transverse serial sections of porcine soleus muscle. ATPase staining. Scale bar, 100 μm. 2a. Preincubation at pH 10.3. 2b. Preincubation at pH 4.5. 2c. Preincubation at pH 4.4. 2d. Preincubation at pH 4.3. 2e. Oxidative capacity visualized by nicotinamide adenine dinucleotide, reduced form (NADH)-dehydrogenase. Five different fiber types—I, I:B, II:A, II:B, and II:C—are identified.

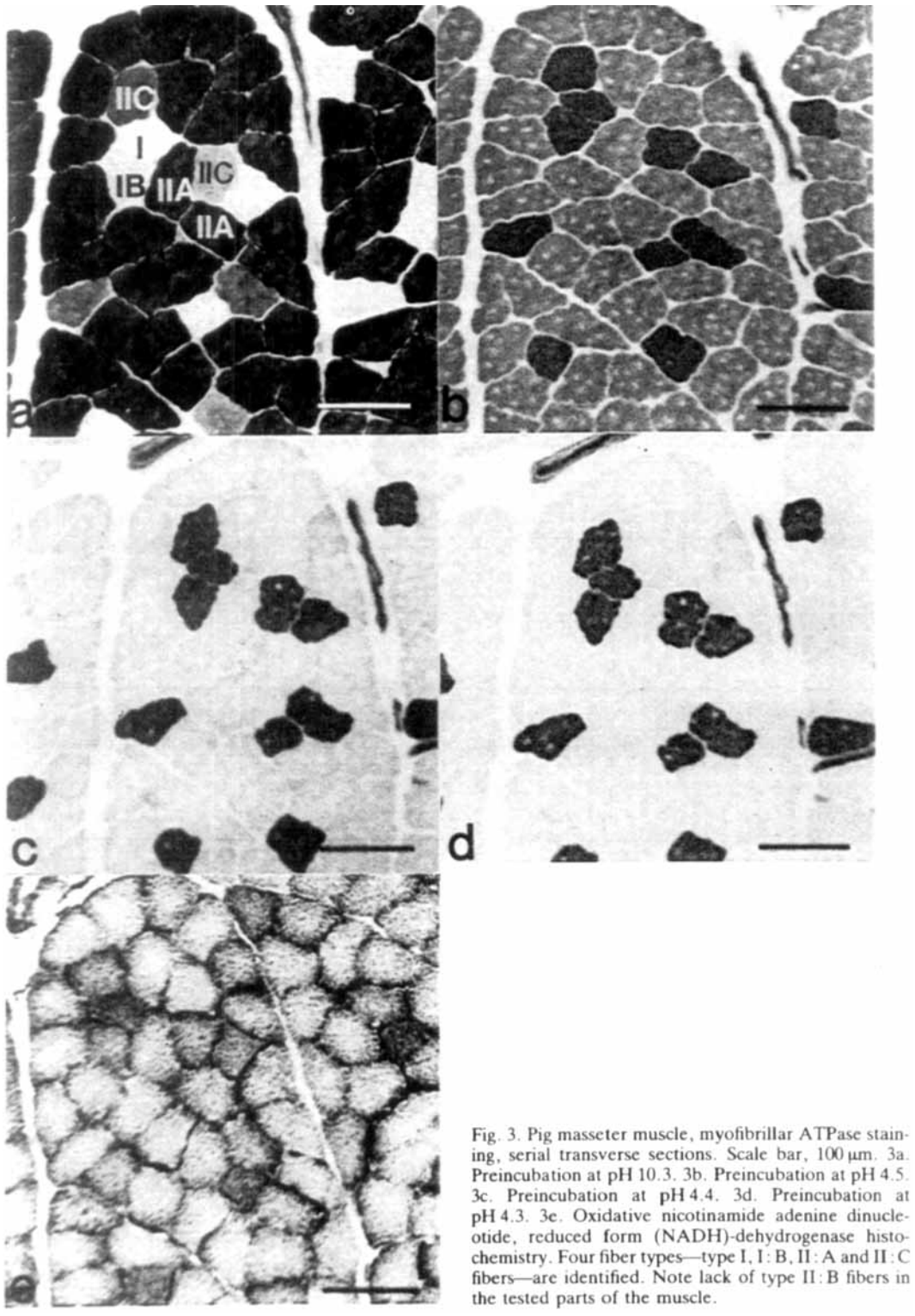

Fig. 3. Pig masseter muscle, myofibrillar ATPase staining, serial transverse sections. Scale bar, 100 μm. 3a. Preincubation at pH 10.3. 3b. Preincubation at pH 4.5. 3c. Preincubation at pH 4.4. 3d. Preincubation at pH 4.3. 3e. Oxidative nicotinamide adenine dinucleotide, reduced form (NADH)-dehydrogenase histochemistry. Four fiber types—type I, I:B, II:A and II:C fibers—are identified. Note lack of type II:B fibers in the tested parts of the muscle.

Table 1. Fiber types in six porcine masseter muscles. Values are relative frequency (%) together with standard deviation (SD)

	No. of fibers	Fiber composition, %			
		I	I:B	II:A	II:C
a*	3221	11.4 ± 6.7	6.2 ± 2.6	78.6 ± 5.9	3.8 ± 2.9
e	2498	18.3 ± 8.5	4.7 ± 1.9	72.7 ± 9.6	4.3 ± 2.3
c	4552	14.3 ± 7.4	6.2 ± 2.7	74.4 ± 9.4	5.1 ± 3.3

* a = antero–intermediate; e = centro–intermediate; and c = postero–intermediate biopsy specimens.

content of type II:A fibers (79%) and the lowest frequency of type II:C fibers (4%). This latter fiber type was most frequently found in the posterior specimen (5%).

When the fiber type distribution was evaluated from biopsy specimens obtained at superficial, central, and deep locations, a clear tendency towards a gradual increase in the proportion of type I fibers with depth and a corresponding decrease in type II:A fibers was observed (Fig. 4). The specimens also contained a higher proportion of II:C fibers near the ramus.

Fiber size measurements

The mean fiber type diameters of four soleus and four masseter muscles are shown in Fig. 5. The fibers in the pig masseter muscle were in general larger (mean, 36 μm; range, 29–47 μm) than the soleus fibers (mean, 28 μm; range, 18–43 μm). The various fiber types in the porcine masseter muscle had a similar size on average. The fiber diameters varied more in the soleus muscle, in which type I:B fiber had a smaller mean diameter than type I:B fibers of the masseter muscle ($p < 0.05$).

Discussion

By means of the preincubation procedure it was possible to separate the three major

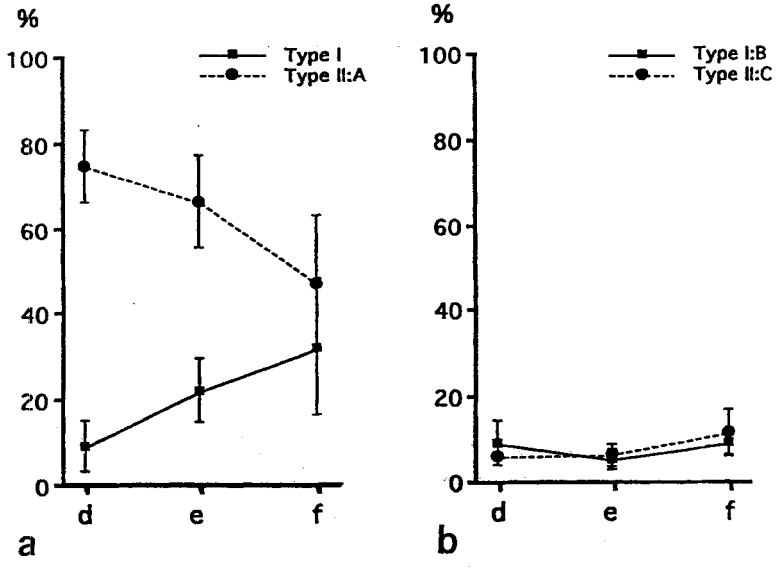

Fig. 4. Relative frequency (%) of fiber types in the central part of four porcine masseter muscles. 4a. Type I and type II A fibers. 4b. Intermediate type I:B and II:C fibers. d = superficial, e = middle, and f denotes deep muscle biopsies.

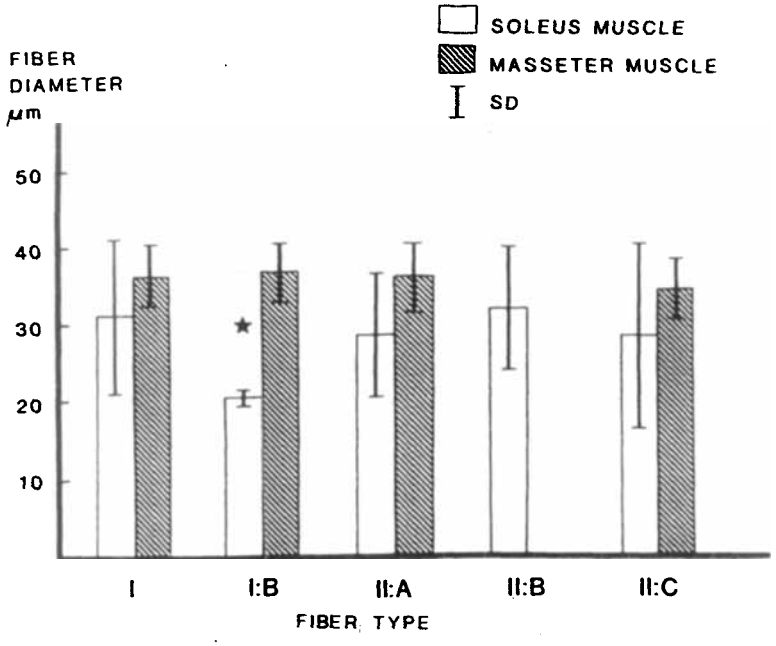

Fig. 5. Fiber diameters (μm) of different fiber types in four soleus and four masseter muscles. *Denotes $p < 0.05$. Note lack of type II:B fibers in the pig masseter muscle.

fiber types (I, II:A, and II:B) and two intermediate fiber types (I:B and II:C) in the porcine soleus muscle in accordance with the original classification system (3). Additional NADH-linked enzyme histochemistry confirmed the expected localization of oxidative capacity in the various ATPase-classified porcine soleus muscle fiber types. Only a minor proportion (10%) of the type II:B fibers diverged in this respect. However, classical type II:B fibers could not be found in the porcine masseter muscle. This observation is based on comparisons of serial transverse-cut, pig masseter and soleus muscle sections after preincubation at pH between 10.3 and 4.3. The porcine masseter type II fiber population had a homogeneous appearance after preincubation at pH 4.4 and 4.5, without disclosing any differences in staining modalities in contrast to the pig soleus muscle. These pH intervals have been reported in the literature to separate type II:A from type II:B fibers (12–14). A comparison of the masseter and soleus sections after preincubation at pH 4.4 and pH 4.5 showed that a medium-stained type II:B fiber reflecting a moderate ATPase activity was not present in the porcine masseter muscle. However, this procedure identified lightly stained type II fibers with weak ATPase reactivity at pH 4.4 and pH 4.5. According to the ATPase-based fiber-typing system this fiber type should be classified as type II:A (3–6).

To minimize methodologic errors in the fiber-typing procedure, sections from the porcine masseter muscle and reference muscle were processed simultaneously in the same preincubation medium during identical conditions (time, temperature) and incubation solution. The sensitivity of the ATPase method and the possibility of bias in the fiber-typing classifications make this approach necessary (14, 15). The porcine masseter muscle diverged in NADH reactivity compared with the soleus muscle. The total masseter muscle fiber population showed a medium intense, equally distributed NADH staining. However, a tendency to strong NADH activity was present in type I and in intermediate type I:B and II:C fibers. It was not possible by this metabolic-based fiber typing to separate any subclasses of type II fibers such as type II:A

and type II:B in the porcine masseter. A high variability in metabolic contents has been found in muscle fibers from animals (42, 43) and humans (44). The high oxidative content in porcine masseter type II fibers supports the observation that these fibers are type II:A. Type II:A fibers have high oxidative content, whereas type II:B fibers have low oxidative capacity (4). The results of this study on fiber types are in agreement with those of Ström (35) and Tuxen & Kirkeby (36) but contradictory to those of Tuxen & Rostrup (37). The diverging results can be explained by the fact that Tuxen & Rostrup (37) were lacking a reference muscle as proper control, which is necessary when functionally specialized muscle groups such as jaw muscles are investigated (48). Furthermore, the authors made statements based on oxidative/aerobic principles without performing any metabolic stainings, such as NADH histochemistry. These two circumstances could explain the diverging results with regard to fiber type classifications.

Our study showed that type I fibers constituted about 15% of the total fiber population, and intermediate type I:B and II:C fibers were found in small fractions of 4–6%. Type II:A fibers composed nearly three-quarters of all fibers. The high frequency of type II fibers is in accordance with the findings of Suzuki (32), Ström (35), Tuxen & Kirkeby (36), and Tuxen & Rostup (37) and is in accordance with the high prevalence of type II fibers found in other pig muscles (45, 46). No significant differences could be found with regard to fiber type and biopsy location. There was a tendency towards a decrease of type II:A fibers and concomitantly an increase in the proportion of type I fibers. A shift towards type I fibers near the bone has been validated for limb muscles such as the vastus lateralis (47). Tuxen & Rostrup (37) found significant differences between fiber type proportions and muscle sample locations. The discrepancies between the studies could be explained by differences in biopsy sites and ages of the investigated animals. The mean fiber type diameters in this study ranged from 29 μm to 47 μm, with an average of 36 μm. No significant size difference was found in the porcine masseter muscle. These results agree with those of Tuxen & Rostrup (37).

Thus, this histochemical investigation showed that the porcine masseter muscle consists of a high relative frequency of type II:A fibers displaying high oxidative capacity. On the basis of a combination of fiber type and physiologic variables (13), the hypothesis could be postulated that the pig masseter muscle would be able to contract for long endurance times before exhaustion. This has been validated by physiologic studies (29, 30).

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