HISTOCHEMICAL CHARACTERISTICS OF THE MASSETER MUSCLE OF TUFTED CAPUCHIN MONKEYS (*Cebus apella* Linnaeus, 1758)

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▪ ABSTRACT: Two samples were removed from the anterior and posterior right masseter muscle of adults male tufted Capuchin monkeys (*Cebus apella*) and submitted to reaction with myosin ATPase (after alkaline and acid preincubation), SDH and NADH-TR. The fibres were classified as fast twitch glycolytic (FG), fast twitch oxidative glycolytic (FOG) and slow twitch (SO) types, although acid reversal reaction of FG-type fibres did not occur. The most abundant fibre type was FG, which also covered the largest area. Since this muscle is voluminous in *Cebus apella*, since the arrangement of its fibres is that of a muscle with powerful contraction, and since FG fibres are also related to muscle contraction force, we may state that this animal has a powerful bite.

▪ KEYWORDS: Masseter muscle; Cebus.

Introduction

Primates have been used as research animals for a better understanding of primitive hominid races, and to obtain data that will help to solve problems affeting the human race. This is possible because of the similarities between simians and man.

Enzyme studies have shown that the composition of masticatory muscle fibres differs from that encountered in other skeletal muscles of limbs and trunk.[5, 14, 36, 37, 40, 46] Masticatory muscles are usually heterogenous in terms of different fibre types. Thus,

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the area and frequency of fibre type vary from species to species to satisfy the functional masticatory requirements of animals with different feeding habits.[2, 36, 37, 38, 44]

McNamara[21] stated that there is a basic similarity between the electromygraphic pattern of the masticatory muscles of Rhesus monkeys and of man. However, Byrd et al.[7] pointed out that some differences in the mandibular dynamics should be considered, since the anatomical arrangement of constituents of the masticatory apparatus is not identical.

Since tufted Capuchin monkeys have been extensively used in experimental studies related to the masticatory apparatus,[23] and since these animals are omnivorous, the fibres of their masticatory muscles are submitted to a functional demand similar to that of man. On this basis, and considering the existence of a basic similarity in the electromygraphic pattern of the masticatory muscles of simians and of man, we believe that a histochemical study of the enzymatic pattern of the masseter muscle fibres of tufted Capuchin monkeys would be of considerable value to man.

Material and methods

Four adult *Cebus apella* males captured in the wild, weighing 2,250 to 4,350 g, were anesthetized with a 1:2 mixture of Ketalar and Rumpum at the dose of 7 to 10 mg/kg body weight. Two samples of the superficial portion of the masseter muscle were removed, one of them from a region near the anterior margin and the other from a region near the posterior margin.

The samples were placed in n-hexane at -70°C (Chayen et al.[8]) for 2 minutes, and then cut into 10 μm-thick sections with cryostat at -20°C. Two series of section were stained with haematoxylin-eosin and Mallory trichrome (Behmer et al.[3]), and were used for the study of the general. Other series of sections were submitted to the reaction for succinate dehydrogenase (SDH) by the method of Nachlas et al.,[22] modified by Wegmann & Tordet-Coidroit,[49] and for nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR) by the method of Pearse,[25] modified by Dubowitz & Brooke.[9] Finally, three other series of sections were submitted to the reaction for myofibrillar ATPase (m-ATPase) at pH 9.4 (Padykula & Herman[24]) after preincubation in alkaline (pH 9.4 and 11.0), and acid (pH 4.3) (Dubowitz & Brooke[9]). Muscle fibres were classified into fast twitch glycolytic (FG), fast twitch oxydative glycolytic (FOG), and slow twitch (SO) (Peter et al.[26]).

Fields selected at random from photographs of the material were examined, and the frequency (%) of the different types of muscle fibres was recorded. The area of the fibre types was also calculated using a digital board coupled to a Unitron AP-II microcomputer. The data were analyzed statistically by the analysis of variance using the F test of Snedecor & Cochran,[42] with the level of significance set at 5%.

Results

The fibres of the masseter muscle presented normal characteristics, except for rare cells in which the nucleus was shifted toward the central region (Figures 1A and 2A). The histoenzymological pattern of the enzymatic reactions was similar in both portions of the muscle (Table 1). Three types of fibres were observed (FOG, FG and SO) arranged in a mosaic pattern (Figures 1B, 1C, 1D, 1E, 2B, 2C, 2D and 2E).

Table 1 – Reactivity of FG, FOG and SO fibres in the anterior and posterior portions of *Cebus apella* masseter muscle

Fibres	Enzymes				
	ATPase 11.0	ATPase 9.4	ATPase 4.3	NADH	SDH
FG	+++	+++	+++	+	+
FOG	++	++	+	++	++
SO	+	+	++	+++	+++

+++ Strong reaction.
++ Intermediate reaction.
+ Weak reaction.

When the data obtained for the AM, PM and GM samples (Table 2) were analyzed by the F and Tukey tests, similar results were obtained. The analysis of variance showed a significant difference in the frequency and in the area of the various fibre types. The Tukey test identified differences in frequency between FG and FOG fibres, and between FG and SO fibres, but did not identify any difference in area among the three fibre types. The "t" test showed no significant difference in area among the various fibre types. This observation, however, seems to be influenced by the elevated standard deviation or by sample size.

Discussion

The fibre population of the masseter muscle of tufted Capuchin monkeys presents a mosaic pattern containing three fibre types, as detected in several animals. The histoenzymological pattern of the reactions permitted us to classify the fibres into FG, FOG and SO.

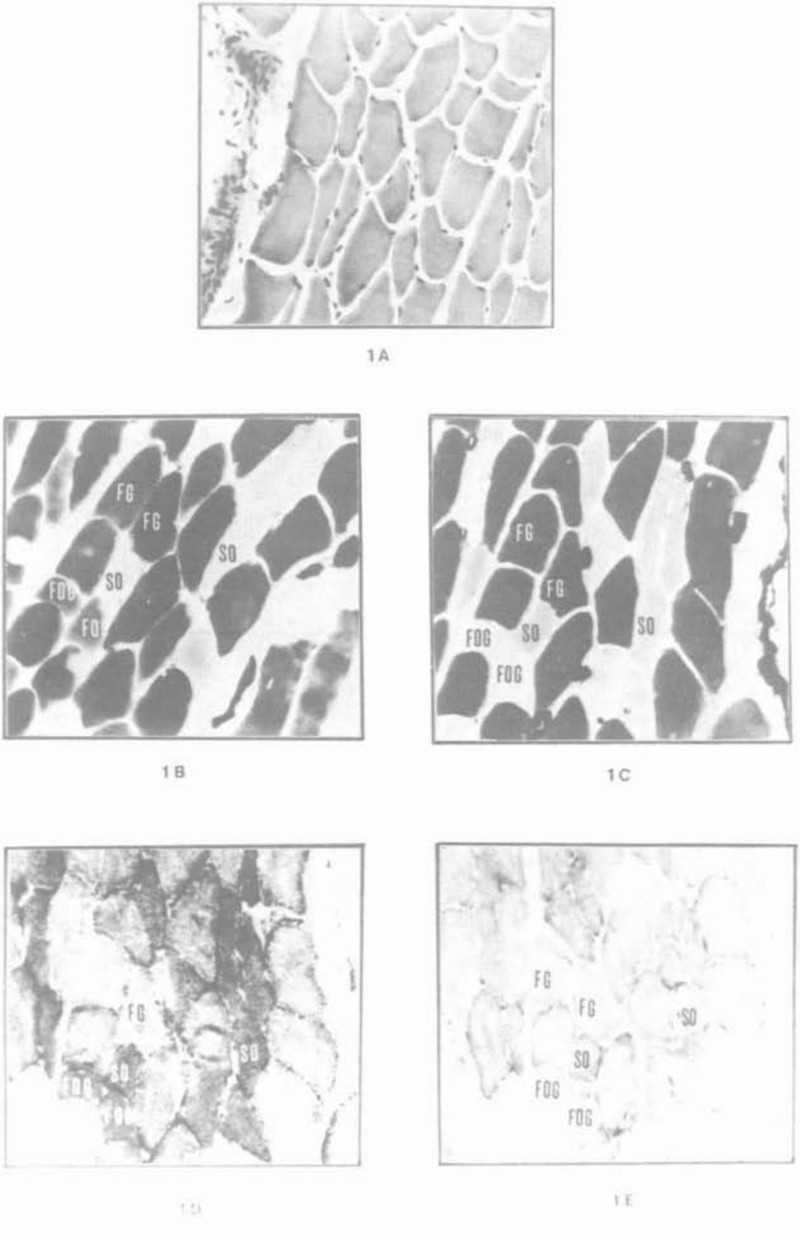

FIGURE 1 – Serial cross sections of the anterior portion of masseter muscle of *Cebus apella*. Muscle fibers Fast Glycolytic (FG); Fast Oxidative Glycolytic (FOG); Slow Oxidative (SO); HE (A); m-ATPase after pH 11.0 (B); m-ATPase after pH 4.6 (C); NADH-TR (D); SDH (E). X200.

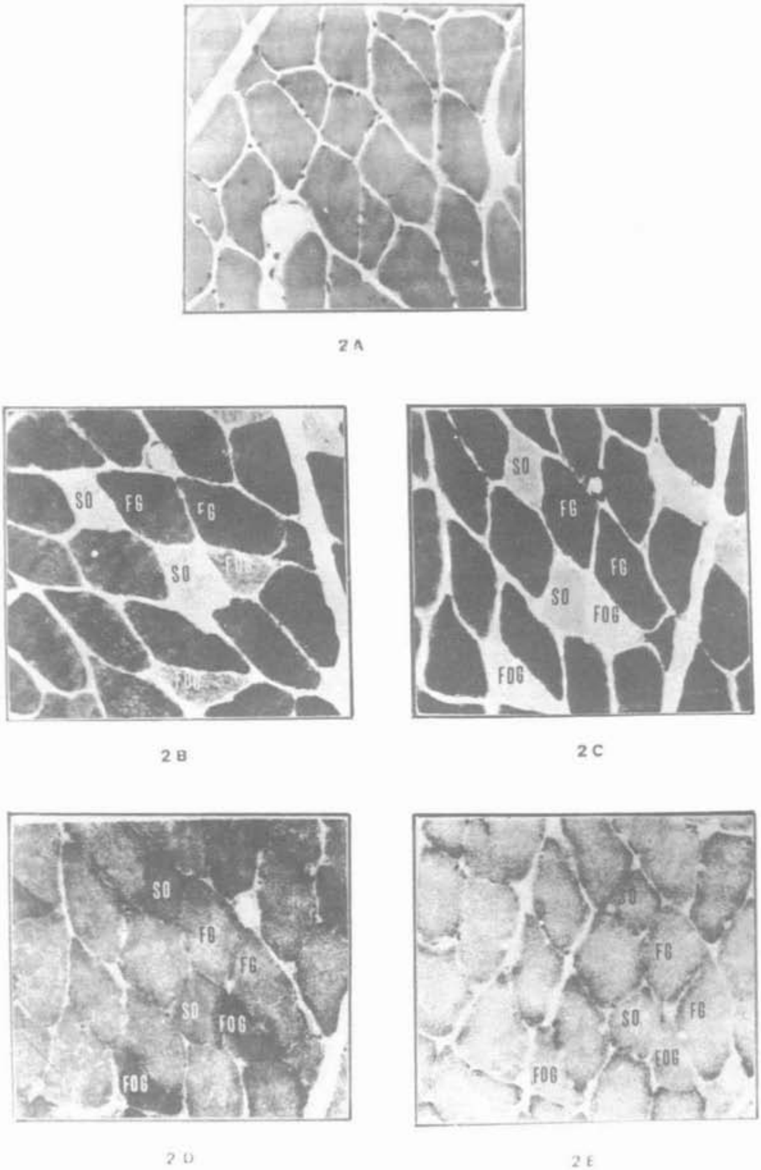

FIGURE 2 – Serial cross sections of the posterior portion of masseter muscle of *Cebus apella*. Muscle fibers Fast Glycolytic (FG); Fast Oxidative Glycolytic (FOG); Slow Oxidative (SO); HE (A); m-ATPase after pH 11.0 (B); m-ATPase after pH 4.6 (C); NADH-TR (D); SDH (E). X200.

Table 2 – Frequency (n), percentage (%), mean area (x), and standard deviation (SD) of different types of fibre detected in *Cebus apella* masseter muscle

Statistic		Fibre types		
		FG	FOG	SO
AM				
	n	78	17.25	14.75
	%	70.90	15.68	13.40
	%	2878.40	1410.89	1384.79
	SD	845.21	423.56	337.06
PM				
	n	82.5	14	13.5
	%	74.99	12.70	12.26
	x	2584.14	1604.54	1531.18
	SD	484.96	341.96	324.96
GM				
	n	80.25	15.62	14.12
	%	72.94	14.19	12.83
	%	2731.22	1507.66	1457.94
	SD	684.15	389.12	330.98

AM = Anterior portion of the masseter muscle.
PM = Posterior portion of the masseter muscle.
GM = Masseter muscle (arithmetic average between anterior and posterior).

Ringqvit,[29] in a study of human masticatory muscles, described a muscle fibre type which she called intermediate or IM, fibre, as also detected by other investigators in the masseter muscle,[11, 39] in the digastric muscle,[10] temporal muscle,[11, 32, 33, 48] and in the masticatory muscles of *Eurinaceus europaeus*.[15] This intermediate fibre type was not detected in the masseter muscle of tufted Capuchin monkeys.

Muscle cells undergo constant changes throughout life in adaptation to changes in functional demand.[30] Thus, fibres of the IM type may represent type I fibres transforming into type II fibres or vice versa, or may be related to impaired function since they have been detected in large amounts in the masticatory muscles of individuals with unilateral mastication. However, generally speaking, simians rarely present malocclusion.[4] On the other hand, the idea that IM type fibres are related to impaired function was ruled out by Ringqvist[31] herself when she observed that these fibres are not present in muscles from limbs with motility disorders. Vignon et al.[48] observed that IM type fibres appear quite early in an individual's life, even before the occurence of a decrease in masticatory function leading to a transformation of type I fibres into type II fibres. Ringqvist[29] stated that additional studies are needed to determine whether type IM fibres are part of the normal constitution or reflectan alteration in muscle function.

FG type fibres from the masseter muscle of tufted Capuchin monkeys do not present acid reversal. A similar fact has been reported by Ringqvist,[33] and Ringqvist et al.,[34] who described the presence of alkali – and acid-stable type II fibres in the human masseter muscle. This type of phenomenon has also been observed in the masticatory muscles of other animals such as rabbits,[38] Rhesus monkeys,[20] and in the masseter muscle of pigs.[45] The absence of acid reversal of fast twitch fibres has also been observed in non-masticatory muscle.[43, 45] These investigators noted that fast twitch fibres presented the same characteristics as those of the type II C fibres reported by Brooke & Kaiser,[6] which are considered to be the precursors of types IIA and IIB, and which are observed in larger amounts in young animals. However, these fibres were detected in adult tufted Capuchin monkeys and Rhesus monkeys,[20] and, therefore, are unlikely to be immature.

Rowlerson et al.[36, 37] described the presence of a fibre type in the masticatory muscles of carnivores and primates which they called IIM. This fibre type presents a very strong acid-stable reaction and a moderate alkali-stable reaction, and reacts only with alpha-IIM serum. For these investigators, this type of muscle fibre is related to the "aggressive" bite, necessary for defense in primates, and for predation in carnivores. Rowlerson et al.[37] propose that the muscle fibres of Rhesus monkeys (described by Maxwell et al.[20]) can be of the IIM type. We believe that the FG fibres detected in the masseter muscle of tufted Capuchin monkeys could be of the IIM type, since they are alkali and acid stable like those of Rhesus monkeys, which also have a powerful bite.

The masseter muscle fibres of tufted Capuchin monkeys with low ATPase activity were different from those detected by Mascarello et al.[18] in the masticatory muscles of some carnivores, which were unaffected by acid or alkaline preincubation.

In the masseter muscle of tufted Capuchin monkeys, the fibre type most often detected in both the anterior and posterior regions was FG, in agreement with data from several previous studies in which the type most often detected was the type II, or fast twitching, or alkali stable.[28, 30, 31, 33, 39] In contrast, this finding differs from those obtained in other studies,[2, 44, 50] in which the most frequent type was the type I, or SO, or slow twitch, or acid stable.

The FG fibres of the masseter muscle of tufted Capuchin monkeys were those with the largest area, in agreement with findings reported by Bosley et al.,[5] Taylor et al.,[46] and Suzuki,[44] who classified these fibres as type II, or alkali stable, or fast twitch. Other investigators, however, reported that type I fibres, or SO or slow twitch, or acid stable, were those with the largest area.[27, 28, 30, 31, 33, 35, 50]

Ringqvist[33] pointed out the differences in percentage and area of the different fibre types between masticatory muscles and limb muscles of some animals, to alert researchers to the possibility of an erroneous diagnosis of myopathy or neuropathy. Preto – Parvis et al.[27] stated that the small size of type II fibres, detected in masticatory muscle samples from individuals with prognathism, recalls the characteristic pattern of atrophy, preferentially involving type II fibres detected in myasthenia gravis.

The small size of type II fibres observed in the masticatory muscles of adult individuals of some species, and especially man, has called the attention of several investigators. Various hypotheses have been proposed to explain this fact. Vignon et al.[48] believe that the type I and II fibres are submitted to identical influences, but type I fibres are not affected by the same circumstances that alter type II fibres. They also propose that the two fibre types may not be subjected to the same influence and may therefore act differently. Preto – Parvis et al.[27] believe that atrophy of type II fibres may be related to the sophisticated diet of modern man, who started to use cooked and sliced food. The lack of atrophy of these fibres observed in tufted Capuchin monkeys supports this hypothesis, since these were animals free in the wild who fed on fibrous food. Since type II fibres are used for energic contractions, in tufted Capuchin monkeys they were not atrophied, as confirmed by their larger area in relation to type I fibres.

On the basis of studies carried out by other researchers, we observed that the percentage and area of the different fibre types in the masseter muscle vary from species to species, reflecting a different functional demand[2, 36, 37, 44] in response to a different use of the masticatory apparatus when feeding or when performing other functions, such as food pressing.

Since the histoenzymological behavior of muscle fibres seems to be related to the type of animal feeding, it must undergo changes with the alterations occurring in feeding in the masticatory apparatus. Some changes are of a transitory nature, as observed when placing metal crowns on the molar teeth of rats to reduce the space of the vetical resting dimension.[1] Others are definitive, as observed when a fine-grained diet is offered,[17] after common carotid occlusion,[13] or in the presence of unilateral mastication.[31]

Differences in percentage and area of the various types of masseter muscle fibres have been observed in animals of the same species. On the other hand, some investigators attribute these differences to age[16, 48] and to sex.[19]

Although some authors have described differences in muscle fibre type between the anterior and posterior portions of the masseter muscle,[11, 12, 14, 19] in tufted Capuchin monkeys this difference was not statistically significant, as also observed by Suzuki.[44] Perhaps we did not find significant differences among fibre types because we removed samples only from the superficial portions of the muscle. Some investigators found differences when they considered the superficial and deep portions of this muscle.[11, 12, 38, 39, 44, 47] The masseter muscle plays a very important role in the mastication of tufted Capuchin monkeys, which is even more important than that of the temporal muscle.[23] This contributes to the powerful bite of the monkey, which can be explained by several factors: the masseter muscle fibres in general are arranged so as to form an angle with their long axis, a fact that gives them power;[41] the masseter muscle of tufted Capuchin monkeys has a very voluminous mass as well as larger numbers and areas of FG fibres which, according to Ringqvist,[30] are directly related to bite force.

Conclusions

On the basis of the present data, we may conclude that the masseter muscle fibres of the tufted Capuchin monkeys present histoenzymological characteristics that permit their classification into FG, FOG, and SO fibres. FG fibres do not present reversal of the m-ATPase reaction at acid pH, present a larger area and are more frequent, followed by FOG and SO fibres. There is no significant difference in percentages and areas of the different fibre types between the anterior and posterior superficial portions of the masseter muscle.

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- *RESUMO: Duas amostras foram retiradas das regiões anterior e posterior do músculo masseter direito de quatro macacos-prego (*Cebus apella*) machos adultos e submetidas a reações com m-*ATPase *(após pré-incubação ácida e alcalina),* SDH *e* NADH-TR. *As fibras foram classificadas como tipos* FG, FOG *e* SO, *embora não tenha ocorrido reação de reversão ácida com as fibras do tipo* FG. *O tipo de fibra mais abundante foi o* FG, *o qual também apresentou maior área. Visto que este músculo é volumoso no* Cebus apella, *que o arranjo das suas fibras é aquele de músculo com contração forte, e que as fibras do tipo* FG *estão também relacionadas com a força de contração muscular, podemos afirmar que este animal tem uma mordida potente.*

- *UNITERMOS: Músculo masseter;* Cebus.

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