Effects of a Soft Diet and Hypothyroidism on the Oxidative Capacity of the Masseter Muscle Fibers of the Young Japanese Field Vole Microtus montebelli

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ABSTRACT—Effects of a soft diet (reduction in mastication activity: an exogenous factor) and hypothyroidism (endogenous factors) on the oxidative capacity of the masseter muscles in the young Japanese field vole *Microtus montebelli*, consisting only of fast-twitch oxidative (FO) fibers in the adult vole, was investigated histochemically and electron microscopically. Oxidative enzyme activity and mitochondrial development in the muscle fibers were not affected by a soft diet, while they were suppressed by doses of propylthiouracil (PTU) (hypothyroidism). Thus, it was suggested that acquisition of the sustained contraction ability in the masseter muscles of the young vole is induced by the endogenous factors rather than the exogenous factor.

INTRODUCTION

Oxidative enzymes of muscle fibers, relating to the sustained muscle contraction, are activated by exercises (overload, training, etc.) (Lowrie *et al.*, 1989; Wernig *et al.*, 1990; Ishihara *et al.*, 1991) or by thyroid hormones (Ianuzzo *et al.*, 1977; Nwoye *et al.*, 1982; Gambke *et al.*, 1983; Bulter-Browne *et al.*, 1984; Sugie and Verity, 1985; Izumo *et al.*, 1986). The effect of thyroid hormones (endogenous factors) on muscle fibers occurs in a short period, though the effect of exercises (an exogenous factor) on them takes a comparatively long time (Maeda *et al.*, 1981a).

The masseter muscles of the Japanese field vole *Microtus montebelli* highly adapt to herbivorous food habit, because they are composed of only fast-twitch oxidative (FO) fibers with numerous well-developed transverse tubules, sarcoplasmic reticula and mitochondria; thus, the masseter muscles are specialized for fast and sustained muscle contraction activity (Sugasawa *et al.*, 1997). As to postnatal development, the vole starts to take solid food at about day 10 and is weaned at about day 20 (Obara, 1975). However, the vole masseter muscles acquired the fast and sustained contraction ability by about day 15 (before weaning), though they are undifferentiated at birth; the masseter muscles of the vole mature more rapidly than that of the rat and mouse (Sugasawa and Mōri, 1997).

From the above, it was suspected that acquisition of oxidative capacity in the young vole masseter muscle fibers needs thyroid hormones rather than mastication activity. The present study was therefore carried out to understand the mechanism of acquisition of the sustained contractile ability in the masseter muscles of the young vole.

MATERIALS AND METHODS

Eighteen newborns of the vole *Microtus montebelli* were kept with their mothers in cages in an environment-controlled room (23 ± 1°C, LD 14:10). These animals were separated into the following groups at birth. At day 15 when all the masseter muscle fibers show the adult-like histochemical reaction (Sugasawa and Mōri, 1997), the young voles in all groups were sacrificed. The control group (8 young) was given *ad libitum* a solid diet (ZF, Oriental Yeast Co., Ltd., Tokyo) and water. The soft diet group (6 young) was given *ad libitum* a fine-grained ZF powder mixed with water in standardized proportion (1:1) and water. The PTU group (4 young) was given *ad libitum* a solid diet and water containing 0.01% 6-η-propyl-2-thiouracil (PTU, Sigma Chemical).

After the body weight was measured, animals were killed by decapitation, and the masseter muscles were removed under ether anaesthesia and rapidly frozen in isopentane solution cooled with dry ice for the light microscopic examination. Thick cross-sections of the muscles (8 μm) were stained for myosin adenosine triphosphatase (ATPase) (Padykula and Herman, 1955) after alkaline (pH 10.5) or acid (pH 4.3) preincubation (Brooke and Kaiser, 1970a, b; Suzuki, 1977) and for reduced nicotinamide adenine dinucleotide dehydrogenase (NADH-DH) (Burstone, 1962). The sizes of the muscle fibers from each group were determined by measuring the maximum distance across the lesser diameter of 50 fibers on photographs (×1,000) in the sections stained for myosin ATPase after alkaline preincubation. The data concerning the body weight and diameter were analyzed by Student's t-test.

Because difference in NADH-DH activity was recognized between the control and PTU groups, ultrastructure of the masseter muscles, histology of the thyroid gland and the serum levels of thyroxine (T4) in both groups were examined. On the other hand, those of the soft diet group were not examined, because difference in it was not found between the control and soft diet groups.

For the electron microscopic examination, the masseter muscles of each group were fixed in 3% glutaraldehyde buffered with 0.1 M

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sodium cacodylate at pH 7.2 for 2 hr, then washed briefly in the same buffer and further fixed for 2 hr in 1% osmium tetroxide buffered with sodium cacodylate at pH 7.2. The tissues were dehydrated in an alcohol series and embedded in Epon 812. Thin sections (~60 nm) were cut on Porter-Blum MT-1 microtome using a glass knife, and doubly stained with lead and uranyl acetate before examination in an Hitachi-H600A electron microscope (75 kV).

The thyroid glands were fixed in Bouin's fluid and sectioned serially at 5 µm, and the sections were stained with hematoxylin and eosin. As to the serum levels of thyroxine (T4), blood in each group (4 examples for the control group; 3 examples for the PTU group) was measured *en bloc* using a SPAC T_4 RIA KIT (Daiichi Radioisotope Laboratory LTD.), because of the small volume of sample available.

RESULTS

There was no significant difference in the mean body weight between the control and soft diet groups and between the control and PTU groups at day 15 (Table 1). Although there was no significant difference in the mean fiber diameter of the masseter muscles between the control and soft diet groups, the mean fiber diameter of the PTU group was significantly smaller (p < 0.01) than that of the control group (Table 1).

For myosin ATPase after alkaline preincubation, all the myofibers of the masseter muscles in the control (Fig. 1A1), soft diet (Fig. 1B1) and PTU (Fig. 1C1) groups strongly reacted. For myosin ATPase after acid preincubation, all the myofibers of the control (Fig. 1A2), soft diet (Fig. 1B2) and PTU (Fig. 1C2) groups weakly reacted.

As to NADH-DH activity, large granular diformazan deposits and strong reaction at the subsarcolemmal region were recognized in the muscle fibers of the control group (Fig. 1A3). NADH-DH activity in the soft diet group showed strong reaction as in the control group (Fig. 1B3). NADH-DH activity in the PTU group appeared coarse and characteristic of the immature fiber with a subsarcolemmal, peripheral zone of hyporeactivity (Fig. 1C3).

Concerning ultrastructural features of the masseter muscle fibers in the control group (Fig. 2A), large mitochondria with closely packed cristae aggregated beneath the sarcolemma. The sarcoplasmic reticula were observed. On the other hand, in the PTU group (Fig. 2B), small and round mitochondria with coarse cristae were found, but they did not aggre-gate beneath the sarcolemma; the sarcoplasmic reticula were observed as in the control group.

The thyroid glands in the PTU group had heightened (columnar) follicular cells, marked reduction of the follicular lumina and colloid content compared with those in the control group (Fig. 3A, B). As to thyroxine hormones, the PTU group displayed lower endogenous levels than did the control group (Table 1).

DISCUSSION

Effects of the soft diet on the masseter muscle

There have been many studies on the development of fiber types of skeletal muscles, and the fiber types have been known to change both structurally and functionally in accordance with an increase or decrease of muscle activities (Maeda *et al*., 1987). Endurance exercise, e.g. treadmill running, is a potent stimulus for adaptation of muscle fiber oxidative capacity. Compared with sedentary animals, muscles of endurance-trained animals have up to twice the capacity to perform oxidative metabolism (Holloszy, 1975; Holloszy and Coyle, 1984).

Masticatory activity is largely carried out by the temporalis, masseter, medial and lateral pterygoid muscles. The masseter muscle is one of the most important muscles for food intake in mammals. In rodents, the masseter muscle is the largest masticatory muscle and it seems to be the most important muscle for biting hard food. It therefore appears that any reduction in masticatory activity caused by a soft diet greatly influences the masseter muscle. It is well known that decrease of the fiber diameter in size and reduction of oxidative enzyme activity in the masseter muscle fibers of the weaned mouse (Maeda *et al*., 1987) and weaned rat (Kiliaridis *et al*., 1988; Miyata *et al*., 1993) fed a soft diet or rat that continued to suckle until day 25 (Maeda *et al*., 1981b) result from relative-disuse atrophy due to the lack of endurance movement of the masticatory muscles that occurs when a solid diet is chewed; thus, both negative effects appear to arise from an adaptive response to contractile function in the masseter muscles of the mouse and rat. However, such effects of the soft diet on the vole masseter muscle fibers were not recognized at day 15, or even at days 20 and 30 (unpublished data). These facts

Table 1. Mean body weight, fiber diameter of the masseter muscles and serum T_4 concentrations in the control, soft diet and PTU groups at day 15 in *Microtus montebelli*

Animal	Body weight*		Fiber diameter*		T_4 µg/dl[†]	
Control	14.32 ± 1.46 g	(8)	10.95 ± 1.14 µm	(8)	6.3	(4)
Soft diet	15.61 ± 1.95 g[NS]	(6)	11.10 ± 0.98 µm[NS]	(6)	–	
PTU	13.06 ± 0.49 g[NS]	(4)	9.23 ± 1.08 µm**	(4)	4.1	(3)

* Values are means ± S.E. for the number of voles indicated in parentheses. The diameters of 50 muscle fibers of the masseter muscles from each animal were measured.
[†] Valuations represent numerical values *en bloc* for the number of young indicated in parentheses. The serum levels of thyroxine in the soft diet group were not measured, because difference in NADH-DH activity was not recognized between the control and soft diet groups.
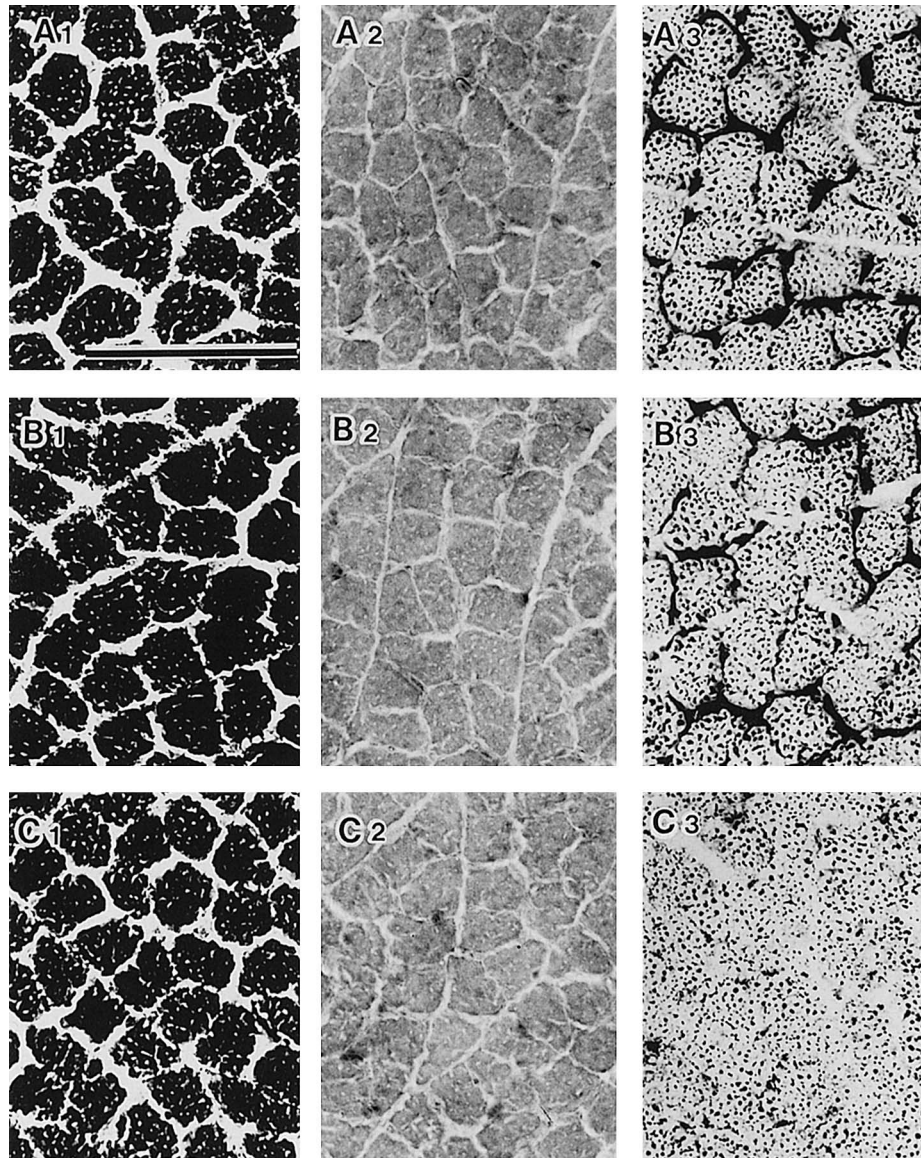[NS] = Not significant; **p < 0.01 vs. controls.

Fig. 1. Histochemical profiles of the masseter muscles in the control (**A**), soft diet (**B**) and PTU (**C**) voles for myosin ATPase after preincubation at pH 10.5 (**1**), at pH 4.3 (**2**) and for NADH-DH (**3**). For myosin ATPase after alkaline preincubation, the control (**A1**), soft diet (**B1**) and PTU (**C1**) masseter muscle fibers strongly react. For myosin ATPase after acid preincubation, control (**A2**), soft diet (**B2**) and PTU (**C2**) masseter muscle fibers weakly react. NADH-DH activity in the control (**A3**) and soft diet (**B3**) masseter muscle fibers have large granular diformazan deposits and show strong reaction at the subsarcolemmal region. NADH-DH activity in the PTU vole appears coarse reaction (**C3**). Bar = 50 µm.

suggested that acquisition of the sustained contraction ability in the vole masseter muscle fibers are not much affected by an exogenous factor (reduction in the mastication activity). Such differences between the vole and murids seem to result from species differences of animals and the muscle functions, as pointed out by Maeda *et al.* (1987).

Effects of hypothyroidism on the masseter muscle

Thyroid hormones are very important in the development of vertebrate skeletal muscles, and an intact thyroid gland is required both for the development of muscle mass and for differentiation of biochemical and contractile characteristics of skeletal muscles (Finkelstein *et al.*, 1991). Hypothyroidism

exerts a pervasive influence on most bodily tissues and organ functions, including the muscular system, where an extensive remodeling of tissue characteristics occurs (Tata *et al.*, 1963). The PTU group was shown by serum hormone measurements to be hypothyroid, as well as by histological properties of thyroid glands. Although a decrease of serum T_4 concentration in the PTU group was less than other studies (Nwoye *et al.*, 1982; D' Albis *et al.*, 1987; Lomax and Robertson, 1992), it is known that such a change affects the histochemical properties of the muscles (Nicol and Johnston, 1981).

Hypothyroidism was associated with decrease in fiber diameter of the masseter muscle in the PTU group. This finding accords with previous observations (Sugie and Verity 1985;
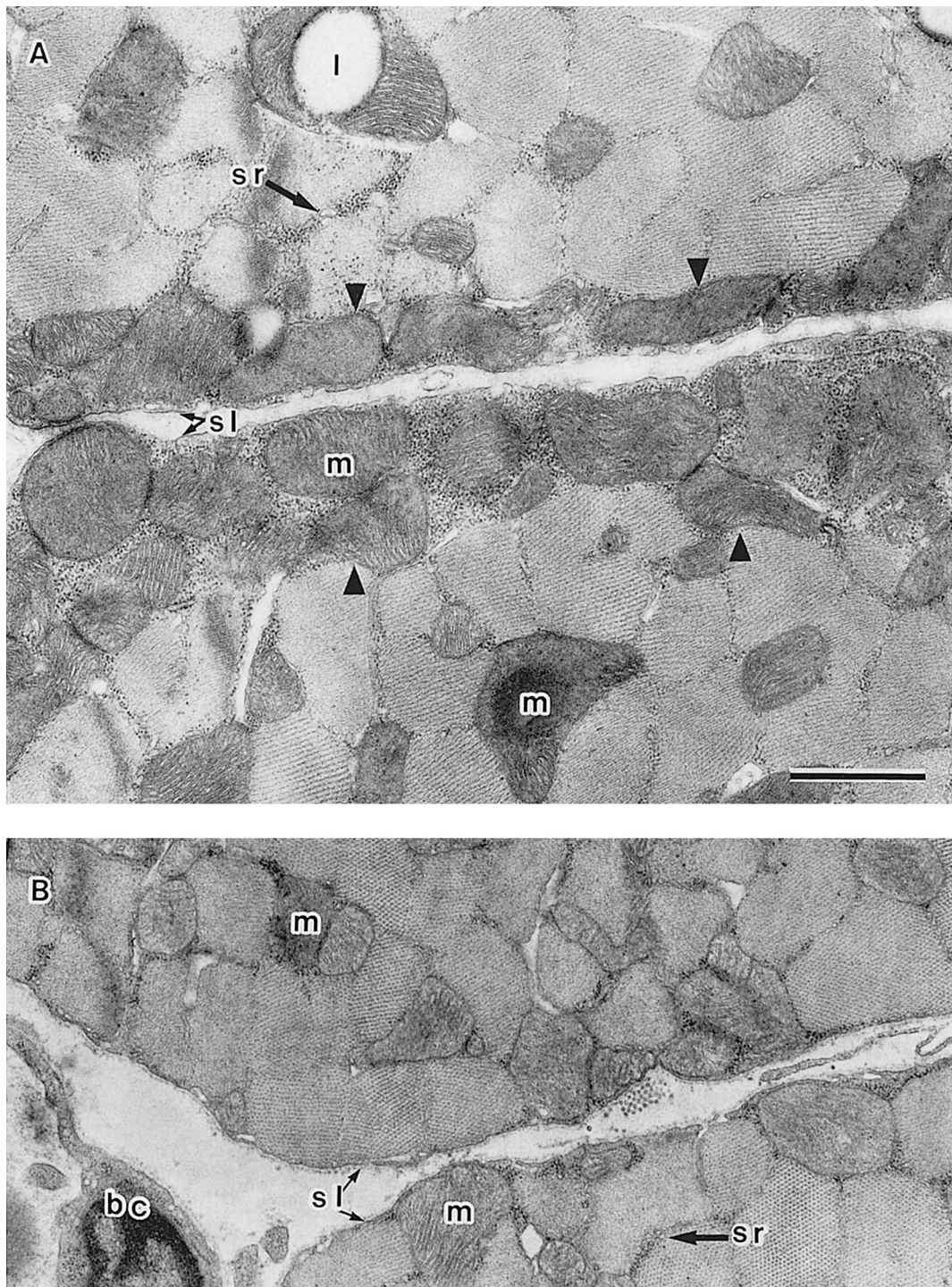
Fig. 2.   Electron micrographs of the vole masseter muscles. (**A**) The control vole masseter muscle fibers having subsarcolemmal aggregation of large mitochondria (arrow-heads) with well-developed cristae. (**B**) The PTU vole masseter muscle fibers lacking in subsarcolemmal aggregation of mitochondria. bc, blood capillary; l, lipid droplet; m, mitochondrion; sl, sarcolemma; sr, sarcoplasmic reticulum. Bar = 1 μm.

Lomax and Robertson, 1992). All the masseter muscle fibers of the PTU group, as in those of the control group, were histochemically classified as fast-twitch fibers according to Peter *et al.* (1972). As to the ultrastructural features of the masseter muscle fibers of the PTU group, some sarcoplasmic reticula seem to directly related to the fast muscle contraction ability

as proposed for other mammals (Ohtsu and Uchida, 1979 in bats; Schmalbruch, 1979 in the cat). It is known that a hypothyroid status induces an actual transformation of fast-twitch fibers to slow-twitch fibers (Mcallister *et al.*, 1991). Our data, however, could not suggest this possibility.

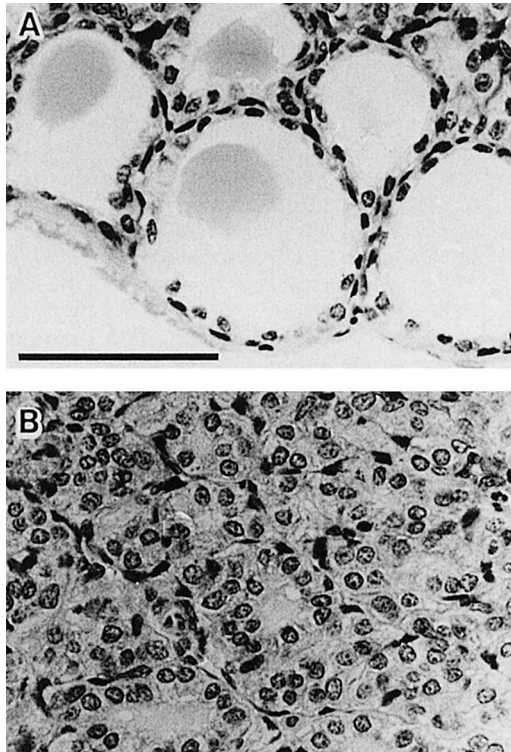It is known that the effects of thyroid hormones on oxida-

Fig. 3. Light micrographs of the thyroid glands in the control (**A**) and PTU voles (**B**). The thyroid gland in the control vole is composed of follicles accumulating large amounts of colloids. The thyroid gland in the PTU vole has heightened follicular cells (marked reduction of follicular lumina and colloid content) compared with the control gland. Bar = 50 μm.

tive capacity of the muscle fiber differ with different muscles or muscle fiber types (Ianuzzo *et al.*, 1977; Janssen *et al.*, 1978; Lomax and Robertson, 1992). In the present study, oxidative enzyme activity of the masseter muscle fibers in the PTU group was weaker than that in the control group. Furthermore, mitochondria of the masseter muscle fibers in the PTU group were less developed than those in the control group and did not aggregate beneath the sarcolemma. Such characteristics of the masseter muscle fibers in the PTU group (day 15) were similar to those of the normal masseter muscle fibers at day 10 (Sugasawa and Mōri, 1997). These findings seemed to imply that the masseter muscle fibers of the PTU group have little sustained contractile ability, and consequently that they have little subsarcolemmal aggregation of mitochondria which supply the energy for the active transport of metabolites through the sarcolemma in this fiber. Furthermore, these facts seemed to imply that the vole masseter muscle fibers belonging to the FO type have many thyroid hormone receptors, and that oxidative capacity, development of mitochondria and their location of these are strongly affected by thyroid hormone concentrations, and therefore that development of the vole masseter muscle (acquisition of the sustained contractile ability) is controlled by thyroid hormones.

In conclusion, it was suggested that acquisition of the sustained contraction ability in the vole masseter muscle fibers (FO type) is affected by endogenous factors (thyroid hormones) rather than an exogenous factor (reduction in mastication activity), and consequently that the maturational pattern of the masseter muscle seems to arise from a predetermined developmental program rather than from an adaptive response to the contractile function.

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