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Myofiber atrophy and histochemical changes in thigh muscles of a lame goat with foot rot

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Summary

Myofibers were classified into three myofiber types (I, IIA, and IIB) by histochemical reactivity for myosin ATPase and NADH tetrazolium reductase (NADH-TR), and the degree of atrophy in myofiber types was examined in the thigh muscles of a lame goat suffering from foot rot. The goat was able to move its affected hindlimb, but did not touch it to the ground or use it for locomotion or standing. In the affected hindlimb, the quadriceps femoris, vastus intermedius, and gluteobiceps muscles were reduced more in wet weight than were the semitendinosus and semimembranosus muscles. The three myofiber types of the semitendinosus muscle atrophied less than those of the other muscle ; they were similar in the degree of atrophy. Type IIA myofibers atrophied more than type I and IIB myofibers in the rectus femoris muscle. Type I myofibers atrophied more than type IIA and IIB myofibers in the gluteobiceps and semimembranosus muscles. The vastus intermedius muscle had only type I myofibers which atrophied greatly. Greatly atrophied type I myofibers varied in the intensity of NADH-TR activity. The activity of menadione-linked glycerol-3-phosphate dehydrogenase increased and 3-hydroxybutyrate dehydrogenase activity disappeared in greatly atrophied myofibers. No transformation of type I into type II myofibers was observed in the muscles. The degree of atrophy in myofiber types of the affected limb seems to be associated with the degree of decline of functional activity.

Introduction

Muscle inactivity and disuse lead to an atrophy of myofibers. A selective atrophy of type I myofibers (1-4) or a moderate atrophy of both type I and II myofibers (5) has been shown in experimentally immobilized muscles of various animals. A selective atrophy of type II myofibers (6) or type I myofibers (7, 8) has been reported to occur in disused human muscles. Type I and II myofibers have been reported to show a similar atrophy in disused human muscles (9). Herbison et al. (10) have suggested that the degree of myofiber atrophy is specific

to the myofiber type and its location in different muscles or muscle regions.

Foot rot is a disease affecting the skin of the junction of skin and hoof matrix in the sheep and goat; it results in lameness. The muscle of an affected limb in a lame goat with foot rot was considered to be voluntarily unused for locomotion and standing. This study was performed to ascertain whether or not a selective atrophy of myofiber types occurred in muscle of the affected limb of the lame goat with foot rot. The histochemical properties of myofiber types were examined in goat muscles.

Materials and Methods

Thigh muscles of a lame goat with foot rot were used in this study. The animal (female; 7 years of age) was Japanese-native Shiba goat and weighed about 30 kg. The goat was pastured, suffered from foot rot, and became lame in the left hindlimb. The goat did not touch its limb to the ground when it stood, but moved its limb back and forth when it walked and ran. The goat used its front limbs and right hindlimb but could not use its affected limb for maintaining a standing posture and walking. It was estimated that more than 2 months passed from the beginning of lameness to slaughter. After slaughter, the quadriceps femoris, gluteobiceps, semitendinosus, and semimembranosus muscles were quickly removed from the left affected limb and the right limb as a control and then weighed. Also the vastus intermedius muscles removed from the quadriceps femoris muscle were weighed. Muscle samples were taken from superficial portions of the belly of the rectus femoris and middle portions of the vastus intermedius muscle as representative of the quadriceps femoris muscle. They were taken from superficial portions of the middle of the gluteobiceps, semitendinosus, and semimembranosus muscles.

The samples were frozen in a mixture of dry ice and acetone and cut serially on a cryostat. Fresh sections (10 μm thick) were incubated for myosin adenosine triphosphatase (ATPase) (11) after preincubation at pH 4.3 and 10.6 (12, 13). Other fresh sections were used for the demonstration of activity of reduced nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR), of menadione-linked glycerol-3-phosphate dehydrogenase (m-GPD) and of 3-hydroxybutyrate dehydrogenase (3-HBD) (14). Some formalin-fixed sections were stained with hematoxylin and eosin.

Myofibers that reacted strongly for ATPase after acid preincubation and negatively or weakly for ATPase after alkaline preincubation were classified as type I, whereas myofibers that reacted negatively to moderately for ATPase after acid preincubation and strongly for ATPase after alkaline preincubation were classified as type II (12). Type II myofibers were subdivided into type IIA with a strong NADH-TR activity and into type IIB with a weak NADH-TR activity. 542 to 1121 myofibers were classified and counted. Diameters of 200 myofibers for

each myofiber type were obtained by measuring the smallest cross-sectional dimension with an ocular micrometer.

Results

Loss of wet weight of muscle

The muscle of the affected limb weighed less than the right control limb (Table 1). The quadriceps femoris and gluteobiceps muscles were reduced more in wet weight than were the semimenbranosus and semitendinosus muscle. The vastus intermedius muscle was reduced the most in wet weight.

Histochemical properties of myofiber types

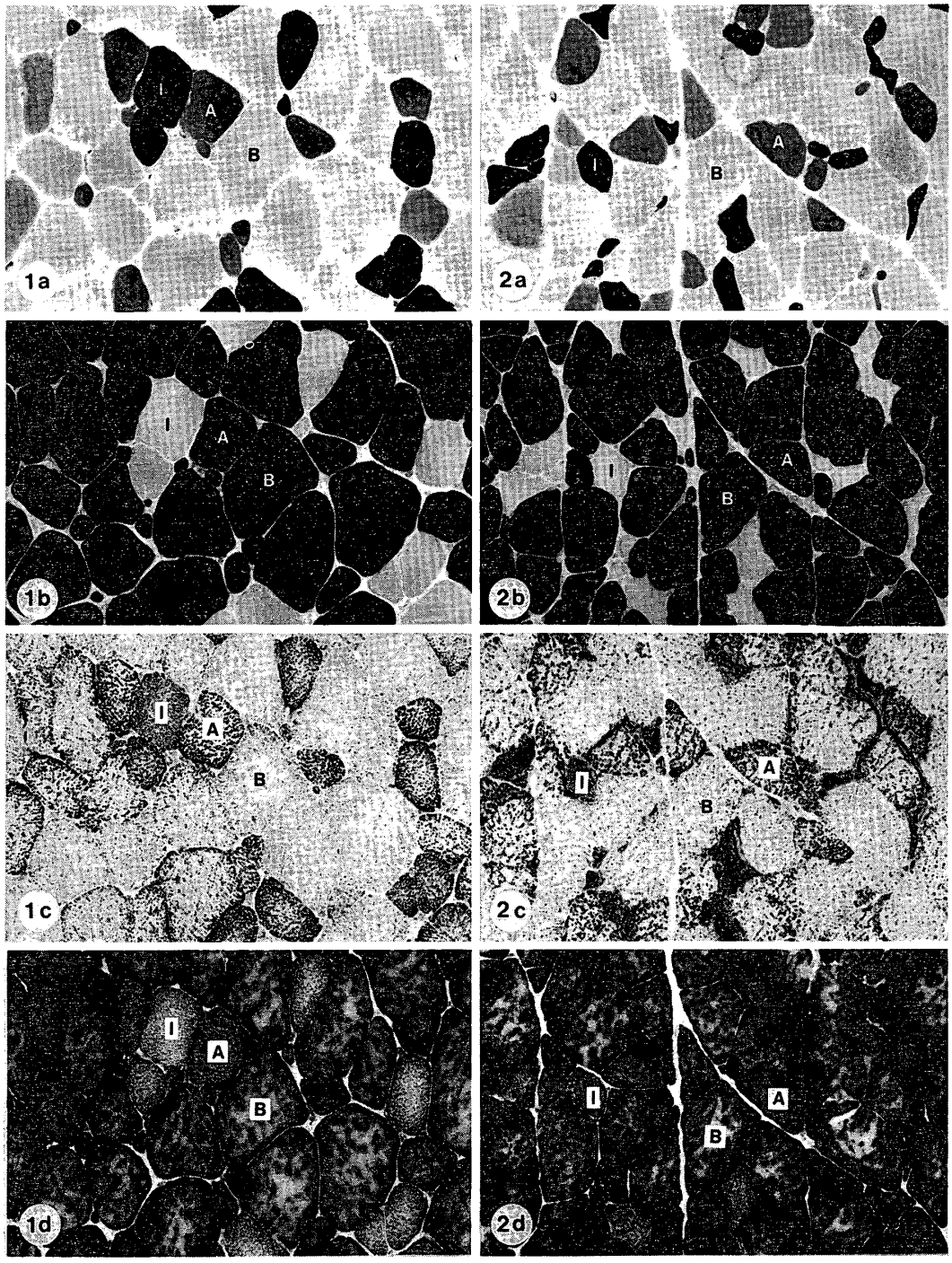
In the control muscles, type IIA myofibers with a strong NADH-TR activity reacted negatively to moderately for ATPase after preincubation at pH 4.3, whereas type IIB myofibers with a weak NADH-TR activity reacted negatively for ATPase after preincubation at this pH (Figs. 1 and 2). Type IIA and IIB myofibers reacted strongly for m-GPD. Type IIA myofibers showed little or no activity for 3-HBD. Type IIB myofibers reacted negatively for 3-HBD activity. Type I myofibers reacted strongly for NADH-TR and weakly to moderately for m-GPD. The type I myofibers of the vastus intermedius muscle reacted moderately for 3-HBD (Fig. 3), whereas those of the other muscles reacted negatively to weakly for 3-HBD. The myofibers that reacted strongly for ATPase after both acid and alkaline preincubation were classified as an intermediate type. The intermediate type reacted strongly for NADH-TR, moderately to strongly for m-GPD, and negatively to weakly for 3-HBD.

In the affected limb, some of greatly atrophied type I myofibers appeared to increase in NADH-TR activity but the other myofibers were unchanged in the muscles except in the vastus intermedius muscle, in which there was a slight decrease in NADH-TR activity (Figs. 1-4). Greatly atrophied type IIA and IIB myofibers increased in m-GPD activity but the other IIA and IIB myofibers were

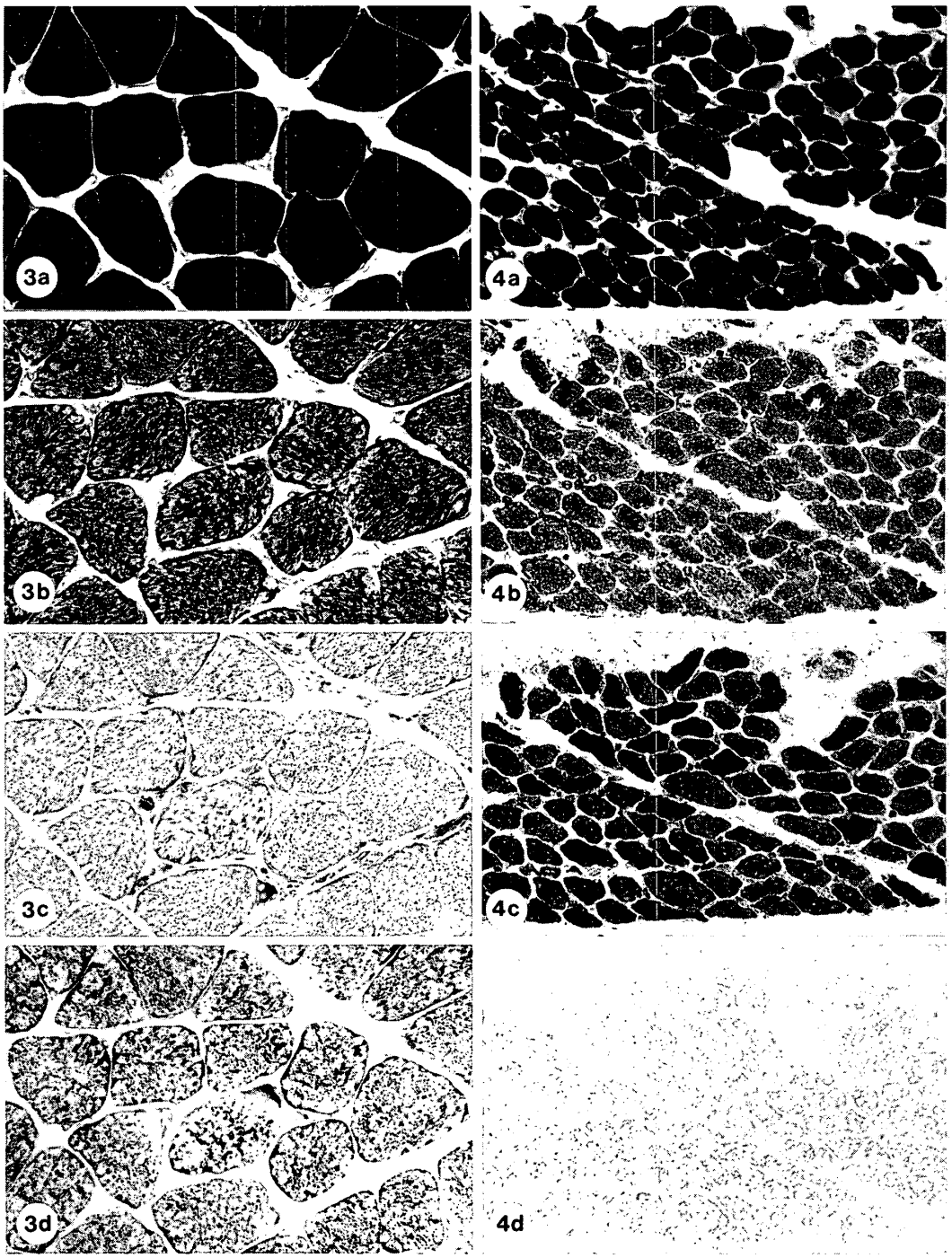
TABLE 1. *Differences in wet muscle weight between the affected and contralateral limbs of a lame goat with foot rot*

Muscle	Contralateral[1)] (gm)	Affected (gm)	Difference (%)
Quadriceps femoris	139	54	−61.2
Vastus intermedius	18	6	−66.7
Gluteobiceps	104	48	−53.8
Semitendinosus	36	24	−33.3
Semimembranosus	82	59	−28.1

[1)] Contralateral muscle as a control



FIGS. 1 and 2. Histochemical properties of myofiber types of the semimembranosus muscle and their atrophy in the lame goat with foot rot. Fig. 1. Contralateral muscle as a control. Fig. 2. Affected muscle. Figs. 1a and 2a. Myosin ATPase reaction after preincubation at pH 4.3. Figs. 1b and 2b. Myosin ATPase reaction after preincubation at pH 10.6. Figs. 1c and 2c. NADH tetrazolium reductase activity. Figs. 1d and 2d. Menadione-linked glycerol-3-phosphate dehydrogenase activity. The muscle has three myofiber types. I, A, and B indicate type I, IIA, and IIB myofibers. All figures are the same magnification. ×260



FIGS. 3 and 4. Histochemical properties of myofibers of the vastus intermedius muscle and their histochemical changes in the lame goat with foot rot. Fig. 3. Contralateral muscle as a control. Fig. 4. Affected muscle. The reactivity for myosin ATPase after acid (Figs. 3a and 4a) and alkaline preincubation is unchanged. Myofibers of affected limb decrease in NADH tetrazolium reductase activity (Figs. 3b and 4b) and increase in menadione-linked glycerol-3-phosphate dehydrogenase activity (Figs. 3c and 4c). 3-Hydroxybutyrate dehydrogenase activity disappears in atrophied myofibers (Figs. 3d and 4d). The muscle has type I myofibers alone. All figures are the same magnification. ×260

unchanged in m-GPD activity (Figs. 1d and 2d). In the semitendinosus muscle, type I myofibers were unchanged in m-GPD activity. In the other muscles, type I myofibers increased in m-GPD activity (Figs. 1d, 2d, 3c, and 4c). Type IIA and IIB myofibers showed no activity of 3-HBD. The 3-HBD activity of type I myofibers was unchanged in the semitendinosus muscle, but disappeared in the other muscles (Figs. 3d and 4d).

Myofiber type composition

In the control, the muscles had more type IIB myofibers than the other myofiber types except the vastus intermedius muscle which had type I myofibers alone (Table 2; Figs. 3 and 4). The semitendinosus muscle had the most type IIB myofibers. The gluteobiceps muscle had fewer type IIA myofibers than type I myofibers, whereas the other muscles had more type IIA myofibers. The semitendinosus muscle had a smaller proportion of type I myofibers than did the other muscles. The proportion of the intermediate myofiber type was the smallest.

In the semitendinosus and rectus femoris muscles of the affected limb, the proportion of type IIA myofibers appeared to increase in association with a decrease in the proportion of type IIB myofibers (Table 2). The proportion of

TABLE 2. *Differences in the proportion and diameter of myofiber types between the affected and contralateral limbs of a lame goat with foot rot*

Muscle	Type	Proportion			Diameter		
		Contra.[1] (%)	Affected (%)	Difference (%)	Contra. (μm)	Affected (μm)	Difference (%)
Rectus femoris	I	17.9	12.9	−5.0	46.7±1.0[2]	30.8±0.6	−34.0
	IIA	27.1	37.1	+10.0	40.0±1.0	21.9±1.0	−45.3
	IIB	53.6	43.8	−9.8	64.3±1.0	49.3±0.6	−23.3
	Inter.[3]	1.4	6.2	+4.8	42.1 ···	30.6 ···	−27.3
Vastus intermedius	I	100.0	100.0	0	63.7±0.7	18.9±0.3	−70.3
Gluteobiceps	I	33.6	37.1	+3.5	40.2±1.0	20.4±0.5	−49.3
	IIA	23.8	25.7	+1.9	32.6±0.8	21.4±0.5	−34.4
	IIB	40.1	36.3	−3.8	48.3±0.8	29.6±0.6	−38.7
	Inter.	2.5	0.9	−1.6	23.9 ···	21.4 ···	−10.5
Semitendinosus	I	6.4	9.2	+2.8	36.5±0.9	28.7±0.7	−21.4
	IIA	22.1	28.2	+6.1	31.3±0.8	26.4±0.9	−15.7
	IIB	71.5	62.6	−8.9	52.3±0.9	40.7±0.8	−22.2
	Inter.	0	0	0	···	···	···
Semimembranosus	I	19.3	18.5	−0.8	31.1±0.8	18.4±0.6	−40.8
	IIA	25.2	27.0	+1.8	31.0±0.8	23.1±0.7	−25.5
	IIB	54.0	54.5	+0.5	46.9±1.0	36.1±0.5	−23.0
	Inter.	1.5	0	−1.5	32.1 ···	···	···

[1] Contralateral muscle as a control
[2] Mean±standard error determined from 200 myofibers
[3] Intermediate type of myofibers

type IIA and IIB myofibers was unchanged in the semitendinosus and gluteobiceps muscles. In the rectus femoris muscle, the proportion of the intermediate myofiber type appeared to increase in association with decreases in the proportion of type I myofibers. The proportion of type I myofibers was unchanged in the other muscles.

Diameters of myofiber types

In the control, type IIB myofibers were larger in diameter than the other myofiber types in the muscles that had three main myofiber types (Table 2). Type IIA myofibers were somewhat smaller in diameter than type I myofibers. Type I myofibers of the vastus intermedius muscle had as large a diameter as type IIB myofibers of the rectus femoris and a larger diameter than the three myofiber type of the other muscles. The intermediate myofiber type was the smallest.

The myofibers reduced in diameter in the affected muscles (Table 2). The degree of atrophy in type I, IIA, and IIB myofibers was similar in the semitendinosus muscle ; the three myofiber types atrophied less than those of the other muscles. Type IIA myofibers atrophied more than type I and IIB myofibers in the rectus femoris muscles. Type I myofibers atrophied more than type IIA and IIB myofibers in the gluteobiceps and semimembranosus muscles (Figs. 1 and 2). Type I myofibers of the vastus intermedius muscles showed the greatest degree of atrophy (Figs. 3 and 4).

Other abnormal myofibers

Neither target myofibers nor targetoid myofibers were found in the muscles. A few myofibers had internal nuclei in the muscles of both limbs. The muscles of the affected limb appeared to have more myofibers with internal nuclei than those of the contralateral limb.

Discussion

Type IIA myofibers are stronger in NADH-TR activity than type IIB myofibers in humans, rats, rabbits (12), pigs (15), cattle (13), and sheep (13, 16, 17). Similarily, the goat type II myofibers were classified into type IIA with strong NADH-TR activity and into type IIB myofibers with NADH-TR activity. In the goat, many type IIA myofibers reacted weakly or moderately for myosin ATPase after preincubation at pH 4.3, whereas all type IIB myofibers reacted negatively for myosin ATPase. The myosin ATPase reaction of type IIA myofibers is completely inhibited by preincubation at pH 4.5, whereas that of type IIB myofiber is resistant to preincubation at this pH in humans, rats, and rabbits (12). The reactivity of myosin ATPase after acid preincubation in goat type IIA and IIB myofibers differs from that in type IIA and IIB myofibers of humans, rats, and rabbits. The intermediate myofiber type falls under the category of

type IIC myofibers of Brooke and Kaiser (12) and corresponds to the intermediate type of pigs (15) and sheep (17).

In the vastus intermedius muscle, the type I myofibers possess a stronger activity of 3-HBD than those of the other muscles. This type I myofiber with large diameters appears to fally under the category of type D myofibers (subtype of type I) of sheep (17, 18) and cattle (19). In the goat, type I myofibers correspond to slow-twitch oxidative myofibers, type IIA myofibers to fast-twitch glycolytic-oxidative myofibers, and type IIB myofibers to fast-twitch glycolytic myofibers (20).

The degree of reduction in wet weight varied in the muscles of the affected limb. Similar phenomena have been reported in unloaded muscles (21) and immobilized muscles (1). The proportion of reduction in diameter of myofibers appeared proportional to that in the wet weight of muscles in the affected limb.

In the affected limb, type I myofibers did not always atrophy more greatly than type IIA and IIB myofibers in the muscles containing three myofiber types, and type IIA myofibers did not always show greater atrophy than type IIB myofibers. Similar findings have been shown in the hindlimb muscles of the bush baby after immobilization. The degree of atrophy is similar in type I and II myofibers in the soleus muscles, but differs between the two myofiber types in the plantaris muscle of the immobilized rat himdlimb (10).

The true muscle disuse induced by tetrodotoxin has been shown to produce a uniform and great reduction in the sizes of all myofiber types (4). The presence of only slightly atrophied myofibers in the affected limb means that motor units composed of them are activated for its movements. Little atrophied type IIA and IIB myofibers presumably function to raise and swing the affected limb while stepping. The great atrophy of type IIA and IIB myofibers suggests that they did not function in raising and swinging the affected limb. The type II myofiber atrophy in the disused human muscle is suggested to be due to a decrease in physical activity (6).

The less atrophied type I myofibers may function to continue raising the affected limb and to swing it. A loss of activity for muscle tonus must induce type I myofiber atrophy (7, 8). The great degree of type I myofibers in the gluteobiceps, semimembranosus, and vastus intermedius muscle suggests that the motor units of the type I myofibers were not activated for maintaining a standing position. The great reduction in wet weight and myofiber diameter of the vastus intermedius muscle indicates that it is a postural muscle.

The intenity of HADH-TR activity in myofibers reflects a capacity to resist fatigue (22). The chronic increase in contractile activity induces an increase in 3-HBD activity in the muscle of the rat (23) and rabbit (24). A loss of activity for the tonus contraction in the affected limb seems to induce a slight decrease in NADH-TR activity in the vastus intermedius muscle and a disappearance in

3-HBD activity in the type I myofibers of the muscles except the semitendinosus muscle.

The increase in m-GPD activity in the atrophied type I myofibers implies that these myofibers shift from dependence on oxidative metabolism to dependence on glycolytic and oxidative metabolism. Similar changes are shown in the immobilized triceps surae muscle of guinea pigs (2) but not in the immobilized muscles of the bush baby (1). Some increases in the proportion of type IIA myofibers in association with slight decreases in the proportion of type IIB myofibers were observed in the semitendinosus and rectus femoris muscle. It is unclear whether the phenomenon is due to a shift from dependence on glycolytic metabolism to dependence on oxidative and glycolytic metabolism in some type IIB myofibers or is caused by a seeming increase in NADH-TR activity due to greater reduction in the myofiber size than in the number or enzyme activity of mitochondria.

A little decrease in the proportion of type I myofibers in association with slight increases in the proportion of the intermediate type suggests that a few type I myofibers were transformed into the intermediate types, which resemble a transitional form in a transformation of myofiber types (15, 17). Such a transitional form has been found in the immobilized muscles of guinea pigs (2). The transformation of type I myofibers into type II myofibers has been shown in the immobilized soleus muscle of the bush baby (1), guinea pig (2), and rat (10), but was not found in muscle inactivity in the lame goat.

The degree of atrophy of three myofiber types differed among the voluntarily unused muscles of the goat with foot rot. The degree of atrophy in the myofiber types seems to be associated with differences in the degree of a decline in functional activity of the myofiber types as suggested by Herbison et al. (10).

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