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The Effect of a High Plane of Nutrition during a Given Period of Growth on Size and Proportion of Skeletal Muscle Fiber Types in the Cattle

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Summary

Three pairs of twin steers were used to study the effect of a high plane of nutrition on the enlargement and proportion of four fiber types in four different muscles. The twins were each divided into two groups: H-group and C-group. The H-group was fed on the diet containing 73–75% concentrate and the C-group on the diet containing 46–47% concentrate from 15–16 to 48–49 weeks of age. Muscle fibers were histochemically classified into four fiber types (A, B, C, and D). The muscle fibers were generally larger in diameter in the 20-month-old animal of H-group than in the one of C-group, but not always in the 14- and 17-month-old animals. It was not shown that only particular fiber types enlarged more in size in H-group than in C-group. The proportion of the B fibers to the total fibers in the three muscles appeared to be greater in H-group than in C-group. There was no consistent increase or decrease in the proportion of the C and D fibers between H- and C-groups. The ratio of the B fibers to the sum of the A and B fibers was generally greater in H-group than in C-group. These findings suggest that the high plane of nutrition during development promotes a transformation of the A fibers into the B fibers to some extent, and that the transformation of the fiber types is limited to the A and B fibers.

Skeletal muscles of the trunk and limb in cattle and sheep are composed of three or four fiber types that differ in the histochemical characteristics (1–4). The type A fibers are higher in NADH-diaphorase activity than the type B fibers. The A and B fibers react strongly for myosin ATPase. The type C and D fibers are higher in NADH-diaphorase activity than the B fibers and weak in myosin ATPase reaction. The D fibers show a distinctive reticular pattern of diformazan deposits in NADH-diaphorase and are larger in size than the C fibers.

The B fibers corresponding to "white fiber" decreased more greatly in fiber size and in the activity of succinic dehydrogenase and NADH-diaphorase than did the A, C, and D fibers similar to "red fiber" in starved sheep (5). These indicate that an extremely low plane of nutrition more highly affects white fibers than red fibers.

The plane of nutrition is one of the most important factors that influence muscle development (6). The growth of the muscle after birth is believed to depend largely on the increase in the length and thickness of muscle fibers. From these, it is presumed that the size and proportion of the four fiber types may be influenced by differences in plane of nutrition during growth. The present study was designed in an attempt to determine whether or not the size and proportion of four types of fibers changed in the calf fed on a high concentrate diet.

Materials and Methods

Six steers (Holstein) that consisted of three pairs of twins were used in this study. They were freely fed on the same diet and in the same environment till 15–16 weeks of age. The twins were each divided into two groups. One group was freely fed on the diet containing 73–75% concentrate in dry matter from 15–16 to 48–49 weeks of age: high concentrate feeding group (H-group). During the same period, the second group was freely fed on the diet containing 46–47% concentrate in dry matter and used as the control group (C-group). From 48–49 weeks of age onwards, all the animals were freely fed on the diet containing 67% concentrate in dry matter, and reared under the same conditions. The first, the second, and the third twins were slaughtered at 14 months old, 17 months old, and 20 months old, respectively. After slaughter, muscle samples were immediately removed from the peripheral part of the belly or center of the *M. longissimus thoracis*, *M. semimembranosus*, *M. semtendinosus*, and *M. triceps brachii* (*Caput longum*). Care was taken to remove these samples from the same portion of each muscle to minimize sampling error. The muscle samples were trimmed, frozen in a mixture of dry ice and acetone, and serially cut in a cryostat at 8 μm thickness.

NADH-diaphroase activity was demonstrated by using the method of Burstone (7). The method of Padykula and Herman (8) was used for the demonstration of myosin ATPase activity. β-Hydroxybutyrate dehydrogenase (β-HBD) activity was demonstrated according to the method of Ogata and Mori (9). Muscle fibers were classified into four fiber types (A, B, C, and D) in the same manner described in the previous paper (4). The muscle fibers showing a distinctive reticular pattern of diformazan deposits in NADH-diaphorase and a moderate activity of β-HBD were classified as the D fibers, because the C fibers were very weak in β-HBD activity in the *M. longissimus thoracis* and *M. semitendinosus* except the *M. serratus ventralis* (4).

Photographs of three portions indicating the same fields in the two sections stained for NADH-diaphorase and for myosin ATPase were taken at 20 magnification and enlarged six-fold. The photographs containing about 450–730 fibers were used for the classification and identification of the fiber types and for counting each of the fiber types. The samllest diameter of 100 fibers of each type was measured with an eyepiece micrometer.

Results

Fiber diameter. In all the muscles of the animals used, the A fibers are generally larger in diameter than the C fibers and smaller than the B and D fibers. The B fibers are larger than the D fibers (Table 1).

At 14 months of age, the C fibers of the *M. longissimus thoracis* and the C and D fibers of the *M. semitendinosus* were larger in the animal of the H-group than in the one of the C-group ($P<0.01$). The B, C, and D fibers of the other muscles were smaller in the animal of the H-group than in the one of the C-group, or did not significantly differ in size between the two animals. The A fibers in the muscles used did not significantly differ in fiber size between the two animals.

At 17 months of age, four fiber types in the *M. semimembranosus* of the animal of the H-group were larger than those of the C-group ($P<0.01$). There was no significant difference in the size of each fiber type in the three other muscles between the two animals.

At 20 months of age, the A, B, and C fibers of the *M. longissimus thoracis* and *M. triceps brachii* were larger in the animal of the H-group than in the one of the C-group ($P<0.01$). The C and D fibers of the *M. semitendinosus* and the four fiber types of the *M. semimembranosus* were larger in the animal of the H-group ($P<0.01$). The A and B fibers of the *M. semitendinosus* and the D fibers of the *M. triceps brachii* did not significantly differ in size between the two animals.

These findings did not show that only particular fiber types enlarged more in size in the animals fed on a high concentrate diet than in the control animals.

TABLE 1. *Diameter of Four Fiber Types in Skeletal Muscles of Steers Fed on Two Different Planes of Nutrition* (μm)

Muscle	Fiber type	14-month-old steer		17-month-old steer		20-month-old steer	
		H*	C*	H	C	H	C
M. longissimus thoracis	A	40.2	41.9	42.3	43.9	53.7	44.3
	B	48.7	51.2	50.5	56.3	65.2	60.8
	C	42.1	36.8	35.8	35.2	43.7	34.7
M. semimembranosus	A	37.8	38.0	46.7	36.8	49.4	44.6
	B	60.8	59.3	67.5	50.1	62.0	55.6
	C	37.3	35.9	44.3	40.0	48.1	36.4
	D	45.5	52.1	51.8	45.1	55.3	48.9
M. semitendinosus	A	46.6	44.6	42.9	46.6	55.2	54.1
	B	65.9	61.1	57.2	54.3	62.2	65.3
	C	49.2	38.8	42.5	42.5	52.1	43.9
	D	62.1	56.0	58.4	56.7	71.7	58.4
M. triceps brachii Caput longum	A	45.3	44.0	43.8	45.2	59.3	45.8
	B	49.2	60.1	54.7	55.7	72.9	64.7
	C	36.4	41.3	44.0	42.7	50.1	45.4
	D	48.0	56.1	47.8	48.9	60.2	61.9

* H: animal fed on 73~75% concentrate diet; C: animal fed on 46~47% concentrate diet

TABLE 2. *Fiber Composition of Four Fiber Types in Skeletal Muscles of Steers Fed on Two Different Planes of Nutrition* (%)

Muscle	Fiber type	14-month-old steer		17-month-old steer		20-month-old steer	
		H	C	H	C	H	C
M. longissimus thoracis	A	32.8	36.6	7.9	30.2	17.6	28.8
	B	52.8	44.7	58.7	54.6	57.6	46.6
	C	14.4	18.7	33.4	15.2	24.8	24.6
M. semimembranosus	A	39.8	42.8	23.6	37.0	31.8	44.2
	B	49.8	47.1	59.1	52.2	51.1	39.7
	C	8.4	7.8	13.9	7.6	14.1	14.8
	D	2.0	2.3	3.4	3.2	3.0	1.3
M. semitendinosus	A	19.8	20.4	14.4	21.8	17.1	22.2
	B	73.6	69.7	79.2	59.5	71.0	65.6
	C	5.7	9.1	4.6	17.9	9.7	10.9
	D	0.9	0.8	1.8	0.8	2.2	1.3
M. triceps brachii Caput longum	A	29.7	41.0	33.7	30.6	29.2	34.9
	B	52.1	34.6	46.8	39.2	42.2	42.9
	C	17.6	23.5	16.3	29.5	28.3	21.6
	D	0.6	0.9	3.2	0.7	0.3	0.6

Fiber composition in muscle. The B fibers were greater in proportion than the other types in all the muscles of the animals used (Table 2). The A fibers were greater in proportion than the C and D fibers in the *M. semimembranosus*, *M. semitendinosus*, and *M. triceps brachii.* The D fibers were the least of the four fiber types of these muscles. In the *M. longissimus thoracis* the D fibers were not found, and the proportion of the C fibers was less or the same to the A fibers.

The B fibers of the muscles except the *M. triceps brachii* were seemingly greater in proportion in all the animals of the H-group than in those of the C-group. Conversely, the A fibers appeared to be less in proportion in the formers than in

TABLE 3. *Proportion of Each of the A and B Fibers to the Sum of These Two Fiber Types in Steers Fed on Two Different Planes of Nutrition* (%)

Muscle	Fiber type	14-month-old steer		17-month-old steer		20-month-old steer	
		H	C	H	C	H	C
M. longissimus thoracis	A	38.3	45.4	12.0	35.7	23.4	38.1
	B	61.7	54.6	88.0	64.3	76.6	61.9
M. semimembranosus	A	44.4	47.7	28.5	41.5	38.4	52.8
	B	55.6	52.3	71.5	58.5	61.6	47.2
M. semitendinosus	A	21.2	22.7	15.4	26.8	19.4	25.3
	B	78.8	77.3	84.6	73.2	80.6	74.7
M. triceps brachii Caput longum	A	36.3	53.9	41.9	43.9	40.9	44.9
	B	63.7	46.1	58.1	56.1	59.1	55.1

the latters. The B fibers were greater in proportion in the *M. triceps brachii* of the 14-month-old animal of the H-group, but not in the 17- or 20-month-old animals.

There was a little difference in the proportion of the C and D fibers between the H- and C-groups, but no consistent increase or decrease in their proportion.

The ratio of the B fibers to the sum of the A and B fibers in the four different muscles is shown in Table 3. The B fibers of the *M. triceps brachii* and *M. longissimus thoracis* in the 14-month-old animal of the H-group were greater in proportion than in the one of the C-group ($P<0.01$). Similarly, the B fibers were greater in proportion in the *M. longissimus thoracis*, *M. semimembranosus* and *M. semitendinosus* of the 17-month-old animal in the H-group and in the *M. longissimus thoracis* and *M. semimembranosus* of the 20-month-old animal in the H-group ($P<0.01$). In the other muscles of each animal in the H-group, the B fibers were seemingly greater in proportion than those in the C-group.

Discussion

The effect of high-plane nutrition on muscle fiber size was not clear in many muscles of the 14- and 17-month-old animals. At 20 months of age, the muscle fibers of the animal fed on a high concentrate diet were larger in size than those of the control animal, but there was no distinct difference in fiber enlargement among the four fiber types. Accordingly, the high plane of nutrition was not shown to influence the enlargement of a particular fiber type. The B fibers were indicated to be most highly affected by starvation (5). From this, it was presumed that the B fibers may be influenced more greatly by a changed plane of nutrition than the three other types of fibers. This assumption was not demonstrated by this study.

Swatland and Cassens (10) reported that the muscle enlargement in high grain line rats was due largely to the hypertrophy of the light fiber corresponding to white fibers. It has been shown that the white fibers of double muscle cattle are greater in size and proportion than those of the normal cattle (11–13). From these facts together with the findings of this study, it is presumed that the enlargement of only a particular fiber type is largely controlled by genetic factors, but little influenced by the plane of nutrition.

The proportion of the B fibers to the total fibers generally appeared to be greater in the animals fed on a high plane of nutrition than in the control animals. An increase or decrease of the C and D fibers was not clear in the two different planes of nutrition. The C and D fibers are definitely distinguishable from the A and B fibers with myosin ATPase reaction. Since the intensity of staining in the myosin ATPase reaction is considered to be directly related to contraction speed of the muscle fibers or the muscle (14–16), the A and B fibers seemed to differ from the C and D fibers in physiological function. The C and D fibers were omitted from the total fibers and the ratio of the B fibers to the sum of the A and B fibers was calculated. As a result, the muscles of the animals fed on a high plane of nutrition showed an increase or that tendency in proportion of B fibers.

Ashmore *et al.* (17) have reported that the αR fibers similar to the A fibers are transformed into the αW fibers corresponding to the B fibers during development. The increase in the proportion of B fibers in the animals fed on a high concentrate diet implies that the high plane of nutrition during development promotes a transformation of the A fibers into the B fibers to some extent.

A transformation of myosin ATPase-high fibers into myosin ATPase-low fibers during development has not yet been demonstrated. The two fiber types are shown to be determined by the nerve supply (18, 19). Therefore, neither A nor B fibers are considered to be transformed into the C or D fibers by changing the plane of nutrition. In this study, the influence of a high plane of nutrition on the transformation of fiber types during development was limited to the A and B fibers and the range of their transformation was narrow.

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