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Number and Area of Muscle Fibers in Mice Selected for High and Low Resistance to Starvation

Takuro OIKAWA, Toshihiro YAMAGISHI and Yutaka MIZUMA

Department of Animal Science, Faculty of Agriculture, Tohoku University, Sendai, Japan

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Summary

The SH and SL mice, selected for high and low resistance to starvation, had different chemical body compositions with high fat and low protein, low fat and high protein, respectively. The characteristics of their muscles under starvation were investigated histochemically in this study. In the control group, there was no difference between SH and SL in body weight, weight of *M. triceps surae* and a cross sectional area of *M. triceps surae*. Comparing the starved group with the control group, the starved SL mcie had less cross sectional area of *M. triceps surae* than the control SL mice, but there was no difference in the muscle of the SH mice. The control SH mice tended to have more red fibers and less white fibers than the SL mice. The control SH mice had more red fibers and less white fibers than the starved SH mice, however, there was no difference in the SL mice. Comparing the average cross sectional area of a muscle fiber between the control and starved mice, the starved SH mice had larger white fibers than the control SH mice, and the starved SL mice had smaller intermediate fibers than the control SL mice in the peripheral part of *M. gastrocnemius*. From these results, it was recognized that the muscles of the SH and SL mice had different characteristics and showed different responses against the starvation.

The characteristics of muscle are considered to be an important subject from the aspect of the efficiency of meat production. The muscles of mice selected for large body size had been extensively investigated, and their relation with body size was clarified. However, the muscles of mice with different chemical body compositions have not been studied well yet.

Suzuki *et al.* (1), (2) revealed that the SH and SL mice, which were selected for high and low resistance to starvation respectively, had interesting characteristics of their chemical body composition, namely, the SH have high fat and low protein contents and the SL have low fat and high protein contents although mice of both lines have almost the same body size. The muscles of the SH and SL mice were examined histochemically and compared to get basic information about the characteristics of muscles in mice with different chemical body composition. Also

the muscles of both lines were investigated under starved condition to compare the response of the muscles between the SH and SL mice.

Methods

Male mice from SH and SL line, whose history was described by Yamagishi (3), were used in this study. Two mice from each of five litters of each lines were picked up randomly, and one was used as a control mouse and the other was used as a starved mouse. The mice were maintained on a commercial diet cube (Funabashi Feed Co. F-1 pellet) and water ad libitum before the experiment. The mice of

TABLE 1. *Body Weight, Muscle Weight and Cross Sectional Area of M. triceps surae in SH and SL Mice.*

Group	Line	Body Weight (g)	Weight of *M. gastrocnemius* (g)	Weight of *M. soleus* (mg)	Cross Sectional Area of *M. triceps* surae (mm^2)
A. Control	SH	28.22±1.97	0.117±0.090	5.1±0.8	17.90±1.61
	SL	28.90±1.25	0.122±0.011	4.6±1.0	17.40±2.60
	t-test SH vs. SL	N.S.	N.S.	N.S.	N.S.
B. Starved	SH	20.32±1.42	0.098±0.005	4.2±0.4	16.27±2.66
	SL	20.68±1.12	0.097±0.005	3.7±0.8	13.38±1.59
	t-test SH vs. SL	N.S.	N.S.	N.S.	N.S.
t-test A vs. B	SH	***	**	N.S.	N.S.
	SL	***	**	N.S.	*

1) Five mice of each strain were used to measure these items in both groups.
2) Mean±S.D.
3) * $P<0.05$, * * $P<0.01$, *** $P<0.001$.

TABLE 2. *Percentage of the Number of Muscle Fibers of Each Fiber Type and*

Gfoup	Line	Peripheral Part of *M. gastrocnemius*		
		Type W	I	R (%)
A. Control	SH	75.24±2.98	11.79±1.86	12.95±1.02
	SL	80.79±9.24	11.00±5.02	8.23±4.37
	t-test SH vs. SL	N.S.	N.S.	N.S.
B. Starved	SH	84.49±3.01	8.24±3.01	7.27±2.09
	SL	81.99±4.97	11.52±3.55	6.49±3.28
	t-test SH vs. SL	N.S.	N.S.	N.S.
t-test A vs. B	SH	**	**	**
	SL	N.S.	N.S.	N.S.

1) All values above are means of measurements of five animals.

both lines in the starvation group were withdrawn from their feed and water for 6 hours a day from 42 to 45 days of age to familialize themselves to the starved situation. Then the mice of both lines in the starvation group were withdrawn from their feed and water for 72 hours from 45 days of age and killed at 48 days of age. A right hind leg was immediately skinned and immersed in aceton with solidified carbon dioxide. The middle part of *M. triceps surae* (*M. gastrocnemius* and *M. soleus*) was excised and made into in situ frozen tissue. After that, the frozen tissues were immediately packed and stored with solidified carbon dioxide in an icebox overnight. The sections (16 μ) were cut with cyostat, and two serial sections of each tissue were stained with hematoxyline-osin (H-E) and succinic dehydrogenase (SDH).

The cross sectional area of *M. triceps surae* was measured on H-E sections. The pictures of SDH sections were taken of *M. soleus* and of two parts of *M. gastrocnemius*, two pictures on a peripheral part and one on a central part. According to Gauthier's classification (3), muscle fibers were classified into red (R), white (W) and intermediate (I) fibers. The percentage of the number of muscle fibers in a unit area was measured for each fiber type. The average area of a muscle fiber was also measured for each fiber type. Muscle weights of *M. gastrocnemius* and *M. soleus* were measured on a left leg.

Results

The averages of the body weight, muscle weight and muscle cross sectional area are presented in Table 1. There was no significant difference in them between the SH and SL mice. Comparing between the control and starved mice, the control mice of both lines had significantly heavier body weight and weight of *M. gastrocnemius* than the starved mice, but there was no significant difference in the

in a Unit area in the Peripheral Part, Central Part of M. gastrocremius M. soleus.

Central Part of M. gastrocremius			*M. soleus*	
W	I	R (%)	I	R (%)
28.36±6.94 36.13±6.16 N.S.	24.03±12.95 30.33±1.81 N.S.	47.62±15.65 33.54± 7.65 N.S.	13.46±7.78 25.82±8.90 *	86.54±7.78 74.18±8.90 *
26.85±3.78 36.43±9.47 N.S.	23.66±8.43 24.69±5.57 N.S.	49.49±9.55 38.88±12.17 N.S.	8.32±4.32 21.28±12.94 N.S.	91.68±4.32 78.72±12.94 N.S.
N.S. N.S.	N.S. N.S.	N.S. N.S.	N.S. N.S.	N.S. N.S.

2) Mean+S.D. 3) * $P<0.05$, ** $P<0.01$.

TABLE 3. *Average Cross Sectional Area of a Muscle Fiber in the*

Group	Line	Peripheral Part of *M. gastrocnemius*		
		Type W	I	R ($\times 10^{-4}$mm^2)
	SH	14.31±1.05	13.65±1.14	9.59±0.38
	SL	17.35±1.24	14.87±1.46	11.50±3.11
	t-test SH vs. SL	**	N.S.	N.S.
	SH	21.49±5.37	15.35±2.77	11.76±2.69
	SL	15.18±1.56	10.44±0.71	8.76±1.54
	t-test SH vs. SL	N.S.	N.S.	N.S.
t-test A vs. B	SH	*	N.S.	N.S.
	SL	N.S.	***	N.S.

1) All values above are means of measurements of five animals.

weight of *M. soleus*. Although the SH mice had no significant difference in the cross sectional area of *M. triceps surae* comparing between the control and starvation group, the starved SL mice had less cross sectional area than the control SL mice.

The percentage of the number of fibers in a unit area for each fiber type is presented in Table 2. The number of the red fibers tended to be more in the control SH mice than in the control SL mice, but there was no significant difference between SH and SL except for the *M. soleus* of control mice, where the red fibers of the SH mice were more than those of the SL mice, and the intermediate fibers of the SH mice were less than those of the SL mice. Comparing between the control and starvation group, there was significant difference in the peripheral part of *M. gastrocnemius* of the SH mice, where the number of the red and intermediate fibers of the starved SH mice was significantly less than the control SH mice and vice versa for the white fibers.

The average area of a muscle fiber in *M. gastrocnemius* and *M. soleus* is presented in Table 3. Comparing between SH and SL, the white fibers of the SL mice were significantly larger than those of the SH mice in the control group. The intermediate fibers of the SL mice were significantly smaller than those of the SH mice in the starvation group. Then comparing between the control and starved mice, the white fibers of the starved SH mice were significantly larger than those of the control SH mice. And the intermediate fibers of the starved SL mice were significantly smaller than those of the control SL mice. In the central part of *M. gastrocnemius* and in *M. soleus*, there was no significant difference between SH and SL, and also between the control and starved mice.

Discussion

The starved SL mice showed a significant decrease in the cross sectional area of *M. triceps surae* compared with the normal SL mice. There was no difference in

Peripheral Part, Central Part of M. gastrocnemius and M. soleus.

Central part of *M. gastrocnemius*			*M. soleus*	
W	I	R ($\times 10^{-4}$mm^2)	I	R ($\times 10^{-4}$mm^2)
19.66±5.52	15.08±5.03	12.26±3.50	14.29±5.97	12.27±4.43
17.35±6.01	13.12±5.07	10.19±2.04	17.01±4.86	14.34±4.94
N.S.	N.S.	N.S.	N.S.	N.S.
18.70±4.33	13.75±1.30	12.43±2.31	15.89±1.71	12.94±1.92
15.44±4.62	10.61±2.05	9.70±2.04	14.51±4.49	12.38±3.52
N.S.	N.S.	N.S.	N.S.	N.S.
N.S.	N.S.	N.S.	N.S.	N.S.
N.S.	N.S.	N.S.	N.S.	N.S.

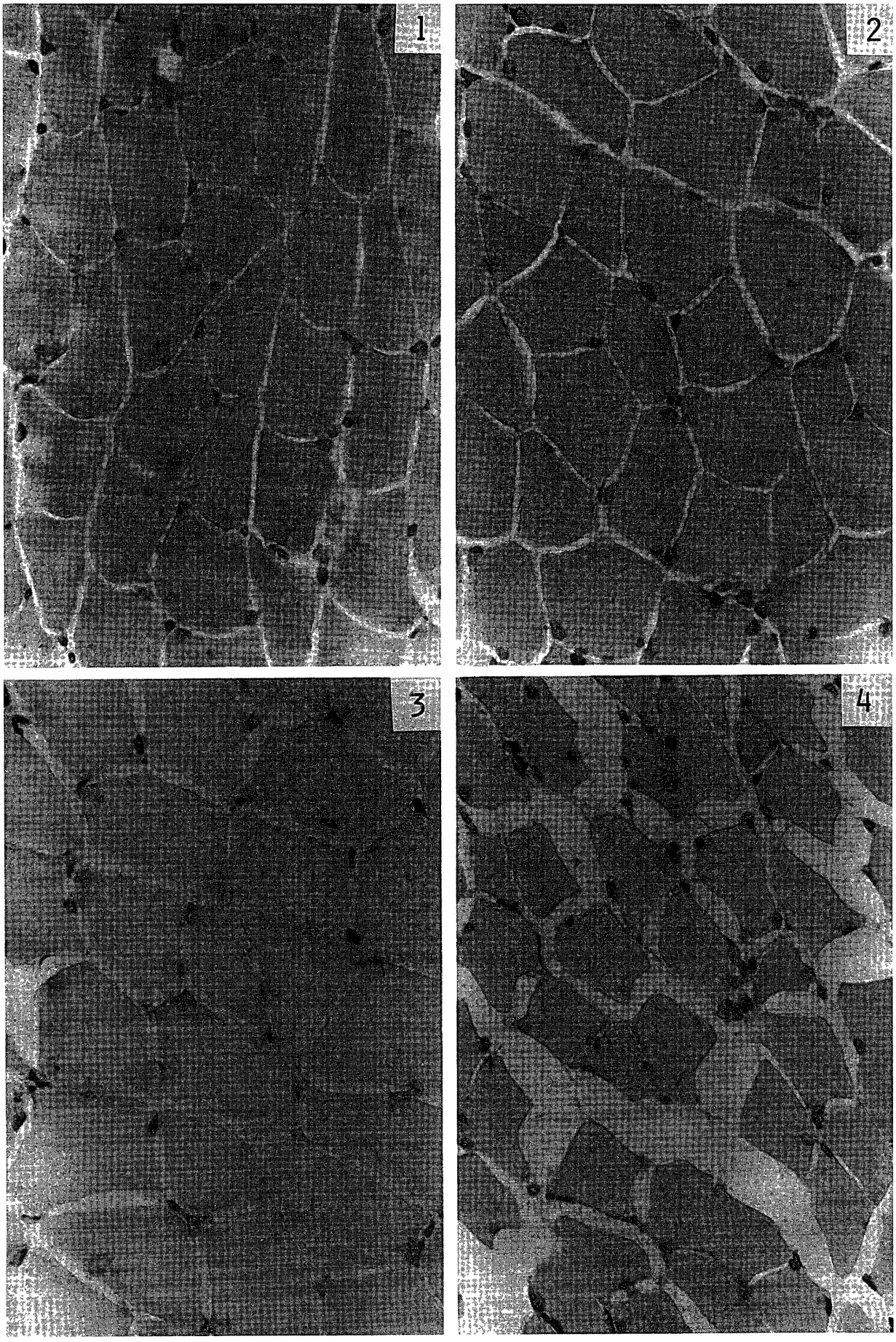
2) Mean±S.D.. 3) * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

body weight and muscle weight between SH and SL of both groups. The rate of the decrease in the cross sectional area of *M. triceps surae* was greater in the starved SL than in the starved SH (23.11% and 9.10%, respectively). The decrease of the cross sectional area of *M. triceps surae* in SL was considered to be caused by the decrease of the average cross sectional area of a muscle fiber. According to Table 3, the decrease of the average cross sectional area of a fiber is greater in the starved SL mice than in the starved SH mice. Particularly in the peripheral part of *M. gastrocenmius*, the response to the starvation was different between SH and SL. The SH mice showed no change or a slight increase in the average area of a muscle fiber after the starvation. However, the average area of fiber in the SL mice decreased under the starvation.

Comparing the effect of starvation on the average area of a muscle fiber between fiber types, there was no obvious difference although Suzuki (5), and Goldspink and Ward (6) indicated that malnutrition affected the white fibers more severely. Comparing the average area of a muscle fiber between *M. gastrocnemius* and *M. soleus*, the peripheral part of *M. gastrocnemius* suffered the effect of starvation more heavily than the central part and *M. soleus*. The different response to the starvation among parts of muscle may come from their different physiological character.

The pictures of the peripheral part of *M. gastrocnemius* of SH and SL are presented at Plate 1. There was no significant difference between SH and SL in the control group. However, fibers of the SL mice were smaller than those of the SH mice in the starvation group. The picture of the muscle fibers in the starved SL mice indicates that the fibers did shrinkage during the starvation. And it is in agreement with the result of the measurement of fiber area.

In the control mice, the number of the red fibers tended to be greater in SH than in SL and vice versa for the number of white fibers. In *M. soleus* of the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control mice, the number of red fibers was significantly greater in SH than in SL, and the number of intermediate fibers was significantly less in SH than in SL. Then comparing the control with the starvation group, the number of red fibers and intermediate fibers was less in the starved SH mice than in the control SH mice, and the number of the white fibers was significantly greater in the starved SH mice than in the control SH mice in the peripheral part of *M. gastrocnemius*. This suggests that the red fibers were changed into the white fibers as described by Goldspink and Ward (6). The SL mice didn't show such a change in the fiber type under the starvation, however, the muscle of SL mice suffered heavy damage under starvation. Thus the SH had more red fibers and responded to the starvation with the change of fiber type. The characteristic feature of the muscle in the SH mice under the starvation may be related to their ability to resist against the starvation and suggest some functional characteristic to survive under starvation.

In the normal situation, the SH and SL mice had almost the same muscle weight, but the muscle composition was different between SH and SL. The SL mice had more white fibers and less red fibers than the SH mice. In *M. soleus*, the SL mice had significantly more intermediate fibers and less red fibers than the SH mice. This indicates that a higher protein content in SL as described by Suzuki et al. (1), (2) doesn't contribute to muscle weight but to the composition of fiber type.

As described above, the muscles of both lines have different characteristics and may have different metabolism related to the resistance to starvation in their muscle, which should be revealed by further work.

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PLATE 1. Cross sections in the peripheral part of *M. gastrocnemius*. All figures are stained by hematoxylin-eosin. ×100.
Fig. 1. Control SH Fig. 2. Control SL Fig. 3. Starved SH
Fig. 4. Starved SL