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Growth Pattern of Muscle Fibers in Triceps Brachii Muscle of Developing Male Chick in Relation to Somatic Development

Mayalata Dimpal*1, Rahul Kundu2

*1Department of Zoology, N. B. Mehta Science College, Palghar, Maharashtra, India

2UGC- CAS Department of Bioscience, Saurashtra University, Rajkot, Gujarat, India

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ABSTRACT

The present study reports the fiber distribution and growth pattern in relation to functional activity and somatic growth rate of the *Triceps brachii* muscle of developing male white Leghorn chick. Three hypotheses were tested in this study; a) on the basis of histochemical properties muscle fibers typing are similar in the muscle mass, b) distribution pattern of different muscle fibers are in relation to the functional activities of that particular species, c) fiber growth pattern are related to the somatic growth rate of that species. Results exposed the existence of all three basic fiber types i.e. red, pink and white which approves the first hypothesis. Results also prove that the growth of all three fiber types is by hypertrophy entirely. True hyperplasia did not marked in any age group; it might be perhaps seized in the late embryonic stage. Splitting of bigger fibers into smaller fibers was observed in some cases of pink and white fibers only. Results showed that all three basic fiber types grew by hypertrophy almost completely irrespective of their position and functional activities. Muscle fibers growth in this muscle mass was absolutely in relation to the somatic growth rate of the chick.

Keywords: Muscle fibers, Growth dynamics; Chick, Triceps brachii, Hypertrophy, Hyperplasi

I. INTRODUCTION

Muscle is one of the extremely specialized and ordered post mitotic tissues. Skeletal muscle is the main contributor to body mass and size in almost all vertebrates. The major components of muscles are muscle fibers which are extremely specialized cells acting as the structural units of skeletal muscle [9]. Muscle fiber number, size, and fiber-type configuration are closely related to each other [27]. These fiber types are identified on the basis of histochemical staining with SDH [21]. Their colour indicates the level of circulation each muscle type receives [16, 17, 20, 19]. In fish musculature fiber types are separated into different muscle masses [33], while mammalian and avian muscles contain a mixture of different fibers types [28]. Studies show that muscle fibers are adapted morphologically and biochemically to fulfill specific functional requirement. Fiber growth by hypertrophy is positively correlated to age and these fiber types may undergo transformations from one fiber type to another depending on functional adaptability [5]. Muscle inactivity is followed by an increase in white fibers which results in muscle atrophy [32]. Many species of birds show annual cycles of atrophy and reconstitution of muscles [4]. In such cases, some muscles are catabolized to produce energy and undergo atrophy as a consequence of inactivity. Studies on fishes have shown that muscles grow either by hyperplasia or hypertrophy [33]. In fishes; muscle growth begins early in their development and continues throughout their life span [31]. The threshold diameter of fibers is genetically fixed after which the fibers start splitting into small fibers [11, 1]. In higher vertebrates the post-embryonic muscle growth is entirely by hypertrophy and the source of additional nuclei are normally derived from myosatellite cells [12]. Skeletal muscle growth in post-hatch birds is determined by hypertrophy and accretion of nuclei which is associated with an increase in the number of nuclei per fiber [28].

II. METHODS AND MATERIAL

Male chick (White leghorn strain, "Broiler"), *Gallus gallus* was selected as experimental animal model. *Triceps* muscle from eight age groups (7 days to 56 days at the interval of 7 days) was selected for the study. A total of 32 animals were sacrificed for the study. Animals were obtained from a poultry farm of Rajkot city and maintained in the departmental animal house facilities in iron cage (36"×24"×24") and in hygienic environments. Growing animals were fed with a poultry starter mash (ingredients-cereal, soybean meal, wheat, grain, corn, pulses) manufactured by Hindustan lever Ltd., and tap water was always made available *ad libitum*. All experiments were conducted according to the ethical norms provided by CPCSEA, India (CPCSEA/CH/RF/ACK-2003).

Required muscles were dissected and mounted on pre-chilled tissue holders and then frozen in cryostat at –18˚C to -20ºC. T.S. of about 10-15μm were cut using Cryostat Microtome and histochemical staining of SDH was performed. Muscle fibers were identified by their physiological and histochemical properties according to the method of Lojda *et. al.,* [20]. Sections were observed under Carl Zeiss Axioscope – II microscope at different magnifications and desired areas were photographed digitally [14]. As large pink fibers and larger white fibers are not always circular in shape, diameter of these fibers was measured at least thrice from three different angles and the mean value was taken. No less than 100 fibers of each fiber type from each possible region were measured from each size class. All morphometrical measurements were done by using the Carl-Zeiss Image Analysis Software and Carl Zeiss Axioscope – II microscope. Collected data were subjected to different statistical analysis like Regression and Correlation Coefficient analysis [29].

III.RESULTS AND DISCUSSION

A. Fiber identification:

Stained sections showed variations in color, shape, size, distribution and orientation when observed under light microscope. The recognition and identification of these fibers were based on staining for the oxidative enzyme SDH. Fibers showed different intensity of colors but belonged to three general divisions i.e. red, pink and white. Fibers increased in diameter during the growth periods. Red fibers appeared more round and smaller as compared to pink and white fibers. Nile blue sulfate staining showed coloration for various kinds of lipids. Phospholipids stained blue whereas, neutral lipids stained as red droplets and were found only in the interstitial spaces. Smaller and nearly round shaped fibers were stained deeply for lipid however larger fibers were stained lightly. Glycogen was stained brilliant red and nuclei stained blue. Lipids were found to be associated more with red fibers while glycogens were found in white fibers only.

Results determined that, the red fibers are the smallest ones and pink fibers are intermediate in size while white fibers are the largest ones [26]. The results of the histochemical experiments showed very strong difference the physiological nature of the three fiber types. The high activity of the oxidative enzyme (SDH) and the low activity of the glycolytic enzyme (LDH) in red fibers indicate the metabolism of this fiber types is mainly aerobic or oxidative which is well supported by high lipid content. Red fibers are therefore associated with aerobic metabolism using lipid and myoglobin as fuel and perform sustained slow contraction [14]. Very little SDH activity and a high LDH activity in white fibers are indicative of mainly anaerobic metabolism through glycolytic pathway using glycogen as fuel and perform fast but rapidly fatiguing contraction. In red fibers high levels of oxidative enzymes like succinic dehydrogenase are present [30]. In pink fibers also oxidative enzyme activity is usually greater [16]. Similar results were obtained in fish myotomal muscles also [25].

B. Fiber Distribution and Orientation:

The relative proportions of red, pink and white fibers in the selected muscle of chick were investigated and the results obtained are presented in (Table-1).

TABLE: 1 DISTRIBUTION OF FIBER TYPES IN TRICEPS BRACHII MUSCLE OF CHICK.

Sr. no	Muscle Name	Fiber types (% frequency)		
		Red	Pink	White
1.	*Triceps brachii*	31.01 ± 0.45	36.24 ± 0.72	32.74 ± 0.36

From the table it appears that the selected muscle showed almost equal proportion of all the fibers i.e. red, pink and white. But still this muscle contains more pink fibers compared to red and white which is almost equal with negligible difference. Chickens have proportionally more pink fibers than others. The muscle fibers were found to be oriented in different manner. Red fibers were found to be concentrated more in the deeper regions near the bone while the white fibers were found abundantly at the periphery. Pink fibers were found to be dispersed erratically within the muscle mass. Histochemical experiment showed the presence of almost equal proportion of red fiber (31.01%) and white (32.74%) fibers. Pink (36.24%) fibers were little higher than red and white hence were major component of the muscle mass. Both pink and white fibers appeared large and somewhat irregular in shape while red fibers were almost rounded in shape. Triceps muscle is mostly composed of pink and white fibers in most vertebrates, which is characteristic of sudden and fast movement for short period. As the experimental animal is flightless bird, hence the distribution of the muscle fibers in this muscle relates with the locomotors action of this muscle.

The selected muscle was found to be composed of all three basic fiber types dominated by pink and white fibers mainly. This suggests an active role of this muscle in the locomotion of the animal. Muscle size is assumed to be proportional to the size of the whole organism hence; growth of the muscle is used as an estimate of whole organism's growth rate. Chickens have proportionally more type II fibers. The different appearances of muscles are due to the presence and predominance of different fiber types in the muscles i.e. the red appearance is due to more cytochrome and myoglobin [3]; while pale muscles are composed predominantly of white fibers with relatively less myoglobin. Intermediate fibers are intermediate in almost all respect. Most muscle of the chickens contain all three types of fiber [2]. Fiber type composition varies according to the type of animal and to the function performed by the particular muscle. Red fibers are designed for slow and sustained body movements hence they are ominously found in postural muscles while the musculature of the forelimbs in larger animals appears to be more involved with maintaining a standing position than in smaller animals.

C. Fibers diameter variation and Growth:

i) Red fibers: The present study showed a distinct variation in different muscle fibers in selected muscle. The diameter of red fibers ranged from 9.19 μm to 28.42 μm in the lowest age class and from 29.73 μm to maximum of 104.14 μm in the highest age class in the developing chick. The mean diameter was 17.72 ± 3.81 μm in the lowest age class which increased to 72.63 ± 13.18 μm in the highest age class. The results show an increase in the fiber diameter from lower to higher age class as the animal grows. Hypertrophy was clearly evident in the red fibers of Triceps muscle (Table – 2).

Table – 2. Mean diameter of red fibers in **Triceps brachii** muscle of Chick. Values expressed are in μm. The muscle is showing gradual increase in mean fiber diameter from the subsequent age classes upto 42 days in all the three fiber types. Thereafter there is decline.

Age	Minimum Diameter	Maximum Diameter	Mean Diameter ± SD
7th Day	9.19	28.42	17.72 ± 3.81
14th Day	14.67	45.60	28.18 ± 5.51
21st Day	24.46	48.44	33.55 ± 4.51
28th Day	17.14	41.83	29.33 ± 4.73
35th Day	34.19	68.72	50.23 ± 7.42
42nd Day	43.90	87.00	63.27 ± 8.40
49th Day	30.70	79.74	60.65 ± 9.48
56th Day	29.73	104.14	72.63 ± 13.18

Result is supported by regression analysis (Fig.2) with high positive correlation coefficient value (R^2 = 0.977). In 35 days there was employment of few small fibers (Fig.1) which might be due to splitting of existing larger fibers or triggering of existing progenitor cells (Fig.2).

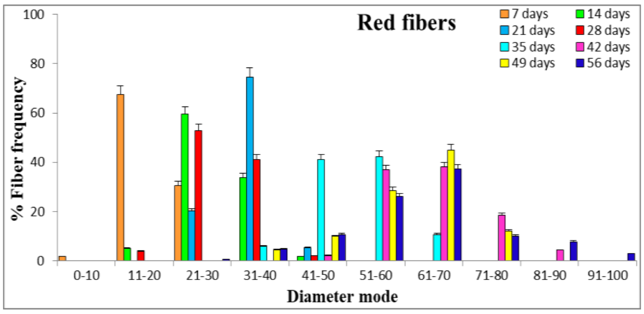

Fig. 1. Graphs showing percent fiber frequencies against diameter modes in **red** fibers of Triceps

brachii muscle of chick. Error bars represent the standard deviation.

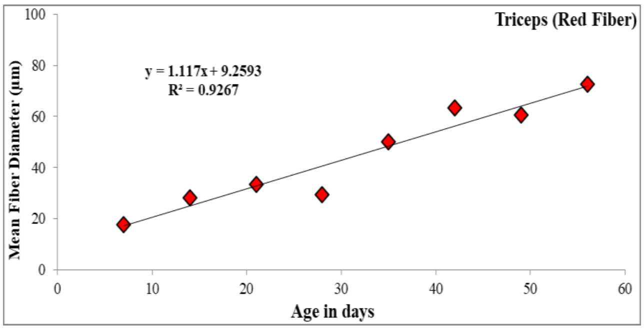

Fig. 2. Regression analysis of **red** fibers of ***Triceps brachii*** muscle of developing chick. Regression equations and Correlation Coefficient values are given.

Present investigation clearly indicates that the main mode of growth of red fibers is mainly by hypertrophy [33]. However, hyperplasia also played an important role in lower age classes [14]. The relative significance of hypertrophy and hyperplasia to muscle growth varies markedly during ontogeny in several vertebrates to muscle growth and growth declines all through the juvenile and the adult, stages and in some species of fishes ceases at around 100% the maximum body length [33, 14, 6].

ii) Pink fibers: The diameter of pink fibers ranged from 10.40 µm to 33.45 µm in the lowest age class and a minimum of 43.38µm to maximum of 127.77 µm in the highest age class in the developing chick (Fig.3). The mean diameter was 18.25 ± 4.24 µm in the lowest age class which increased to 75.76 ± 14.32 µm in the highest age class. The results clearly show an increase in the fiber diameter from lower to higher age class as the animal grows (Table – 3).

Table – 3. Mean diameter of pink fibers in **Triceps brachii** muscle of Chick. Values expressed are in µm. The muscle is showing gradual increase in mean fiber diameter from the subsequent age classes upto 42 days in all the three fiber types. Thereafter there is decline.

Age	Minimum Diameter	Maximum Diameter	Mean Diameter ± SD
7th Day	10.40	33.45	18.25 ± 4.24
14th Day	14.37	40.92	29.77 ± 4.34
21st Day	25.06	42.26	32.66 ± 3.67
28th Day	19.72	47.69	31.44 ± 6.25
35th Day	25.53	75.36	52.73 ± 8.26
42nd Day	51.95	104.27	73.93 ± 11.04
49th Day	31.82	88.57	65.64 ± 8.32
56th Day	43.38	127.77	75.76 ± 14.32

Age and mean fiber diameter have strong positive correlation (R^2 = 0.991) while fiber frequency and age have negative correlation (Fig.4).

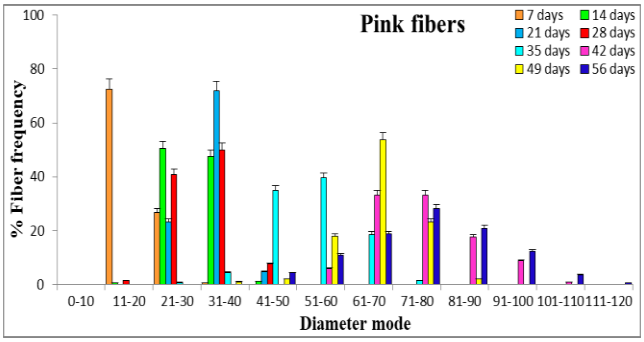

Fig. 3. Graphs showing percent fiber frequencies against diameter modes in **pink** fibers of Triceps brachii muscle of chick. Error bars represent the standard deviation.

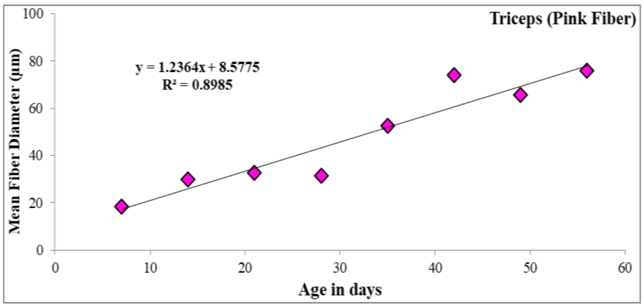

Fig. 4. Regression analysis of **pink** fibers of ***Triceps brachii*** muscle of developing chick. Regression equations and Correlation Coefficient values are given.

A gradual shifting of fiber frequency values towards next higher diameter modes was evident in this muscle (Fig.3&4)) which is indicative of growth by hypertrophy (Table – 3). Small new fibers observed at higher age group are may be the result of budding and splitting of larger fibers (Fig.3). Pink muscle appears to be intermediate between red and white muscle [18, 6].

iii) White fibers: The diameter of white fibers ranged from 8.26 µm to 36.12 µm in the lowest age class and a minimum of 40.25 µm to maximum of 95.39 µm in the highest age class in the developing chick. The mean diameter was 20.94 ± 5.22 µm in the lowest age class which increased to 70.05 ± 12.24 µm in the highest age class (Fig.5). The results evidently display an increase in the fiber diameter from lower to higher age group as the animal grows (Table – 4).

Table – 4. Mean diameter of white fibers in **Triceps brachii** muscle of Chick. Values expressed are in µm. The muscle is showing gradual increase in mean fiber diameter from the subsequent age classes upto 42 days in all the three fiber types. Thereafter there is decline.

Age	Minimum	Maximum	Mean

	Diameter	Diameter	Diameter ± SD
7th Day	8.26	36.12	20.94 ± 5.22
14th Day	17.24	40.96	30.31 ± 3.41
21st Day	24.50	51.39	33.06 ± 4.57
28th Day	21.57	54.36	34.11 ± 6.37
35th Day	35.78	71.61	52.46 ± 7.82
42nd Day	51.48	106.70	80.39 ± 10.56
49th Day	39.02	79.24	62.76 ± 7.81
56th Day	40.25	95.39	70.05 ± 12.24

There is perfect correlation between age and growth (R^2 = 0.980). Diameter modes were increased successively all through the age groups (Fig.6).

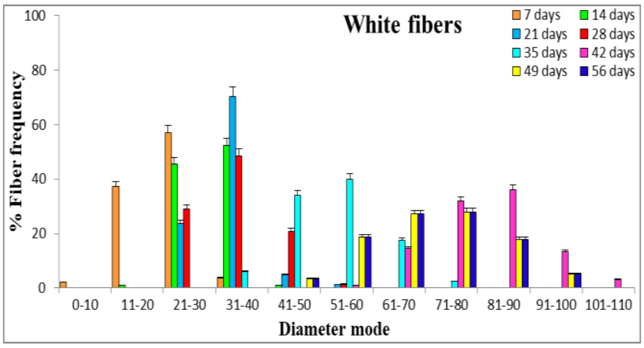

Fig. 5. Graphs showing percent fiber frequencies against diameter modes in **white** fibers of Triceps brachii muscle of chick. Error bars represent the standard deviation.

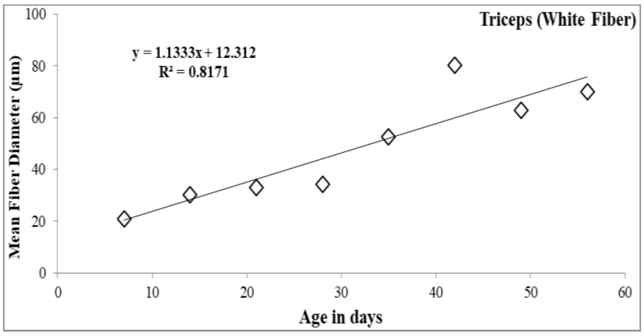

Fig. 6. Regression analysis of **pink** fibers of ***Triceps brachii*** muscle of developing chick. Regression

equations and Correlation Coefficient values are given.

It appears from the results (Fig. 5); recruitment of small new fibers was observed in high frequency in the lower age group and some recruitment was also observed in extremely higher age groups. In higher age groups, shifting of modal frequency values towards higher diameter modes was prominent. This is revealing of an rise in fibers having medium diameter, which is formed by splitting of prevailing large fibers that reached to a theshold diameter [11, 15].

Present investigation revealed that the modes of growth of red, pink and white fibers are mostly by hypertrophy only. Though the frequencies of certain intermediate diameter modes were high in nearly all age groups. Furthermore, the white fibers exhibited an extended range of fiber diameter with highest diameter modes in all three fiber types which is indicative of the splitting of bigger fibers into smaller ones [22]. The addition of persistent myoblasts or myosatellite cells [13, 10] also attributed towards the overall growth and development of muscle fibers in the selected muscle [8, 7]. As it appear from present investigation that the growth dynamics of all three fiber types in the Triceps brachii muscle is by hypertrophy exclusively [25, 6]. The recruitment of small new fiber is not at all evident. The growth dynamics of this muscle is typically by muscle fiber diameter only.

Increase in red and pink fiber area in physically active individuals has been reported earlier in mammals [5] and birds [24, 4]. Thus, the increased hypertrophy of the fibers from 28 days of age in the selected muscle of broilers may be related to higher muscle activity during the experiment period i.e. the broilers interacted more with each other. Our results showed increase in the diameter of the red, pink and white fibers only until 42 days of age. This absence of hypertrophy from 42 to 56 days of age for the three fiber types specify that the muscle fibers reached their determined growth at 42 days but why the hypertrophic growth of the muscle fibers stopped during 42 to 49 days of age remains to be understood.

IV.CONCLUSION

Present study discovered the presence of all three basic fiber types i.e. red, pink and white in the selected muscle. The growth of the muscle was found to be exclusively by hypertrophy only; which increases the muscle mass by increase in diameter of the fiber type. As the selected animal is one of the major agricultural animal the data can be used to improve the quantity as well as the meat quality of the animal.

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