

## LEVELS OF TOTAL LIPIDS IN STRESS-INDUCED CHICK SKELETAL MUSCLE

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Lipids are the major fuel for slow but sustained functioning of the narrow red fibers. Therefore, total lipid content of chick *Gastrocnemii* (pars *lateralis*, *medialis* and *intermedia*) muscle has been studied under normal, denervated and exercised conditions from 1-56 days of post hatching growth period. For each investigation, atleast 4 muscle sets were employed. The total lipid content in all the *Gastrocnemii* ranges from 0.962–29.923 mg/gm under normal, denervated and exercise stress. The pars *lateralis* shows an immediate response to the ablation of neural control when a very steep rise in its lipid content is recorded between 3-14 days post denervation. Thereafter, a decline in lipid levels is noticed. Under exercise stress, *G. intermedia* behaves much like the *G. lateralis* when it depicts relatively high lipid contents between 1-7 days stage. Exercise stimulates lipogenetic processes as well as rate of lipid mobilization in the muscles but prolonged exercise retards lipid mobilization.

**Key words:** *Gastrocnemii*, Sciaticotomy, Chick skeletal muscle.

### Introduction

Skeletal muscle is the largest energy generator and energy consumer in the animal body. Both carbohydrates and fats serve as the major energy sources and the two metabolites constitutes the fuel reservoir in all the muscles. Biochemical investigations on skeletal muscle lipids have been limited though extensive investigations in the lipids in various other tissues like aorta, heart, liver, skin, passage cells, brain and sclera etc. have been carried out by numerous workers [1-6]. It is well known that lipids serve as the most concentrated source of energy to the tissue [7]. The energy yield of lipids is almost double that of carbohydrates [8]. Lipids play very important role as structural components in the muscle fibers. They contribute to the structure of sarcolemma, sarcoplasmic reticulum and mitochondrial membrane system [9]. Even at rest, the lipids remain a major energy substrate in the muscle [10-11]. During diseased states in skeletal muscles, changes in serum phospholipids and serum cholesterol have been reported [12-14]. Little information is available regarding the lipid content of chick skeletal muscle under conditions of denervation and exercise, therefore, this investigation is carried out.

### Materials and Methods

One day old chicks (200) were obtained from Government poultry farm, Sundernagar, H.P. These were maintained in poultry pens in the animal house under normal hygienic conditions. Food and water were given *ad libitum*. The chicks were divided into two groups whereas the animals in the first group serves as controls, the animals of the 2nd group were subjected to unilateral sciaticotomy on the 5th day of their post-hatching development [15]. As a result, the entire body weight is borne by the contralateral limb which is subjected to continuous workoverload stress. Therefore, the group of de-

nervated animals provided two experimental designs with regard to distinctly separate stress conditions. The three *gastrocnemii* (pars *lateralis*, *intermedia* and *medialis*) muscles of *Gallus domesticus* (white leg horn variety) were used for estimation of total lipids at 1,3,5,7,14,21,28,35,42,49 and 56 days stages. At each stage, 4 animals were sacrificed by cervical dislocation and equal number of muscle sets (normal, denervated and exercised) were employed at any stage of investigation.

The lipid extraction was done according to the method of Folch [16]. The quantitative estimation of total lipids was done by the acid-dichromate method of Panday [17]. Controls were also run alongwith statistical analysis. The optical density was read at 590 nm in Bausch and Lomb spectronic-20. The standard calibration curve was drawn using various concentrations of palmitic acid.

### Results and Discussion

The results of quantitative estimation of total lipids of normal, denervated and exercised muscles of chick are presented in Table 1, Fig. 1.

During the present investigation, the three *Gastrocnemii* exhibit increasing lipid levels during 3-28 days of their normal growth but towards the later period a relative decline is experienced. Passing through a period of gradual lipid depletion between 28-42 days, the muscle *G. intermedia* finally records  $12.318 \pm 3.058$  of lipids at 56 days stage. It is pertinent to point out that amongst the three *Gastrocnemii*, *G. intermedia* attains the highest lipid level of  $19.926 \pm 3.439$  at 5 days post hatching. The lowest lipid levels in all the muscles are recorded at 42 days stage. The data clearly shows that the *G. intermedia* maintains the highest total lipid content during 1-21 days, as the muscle is predominantly white in nature [18]. Therefore, it is evident that the lipids in this muscle are the

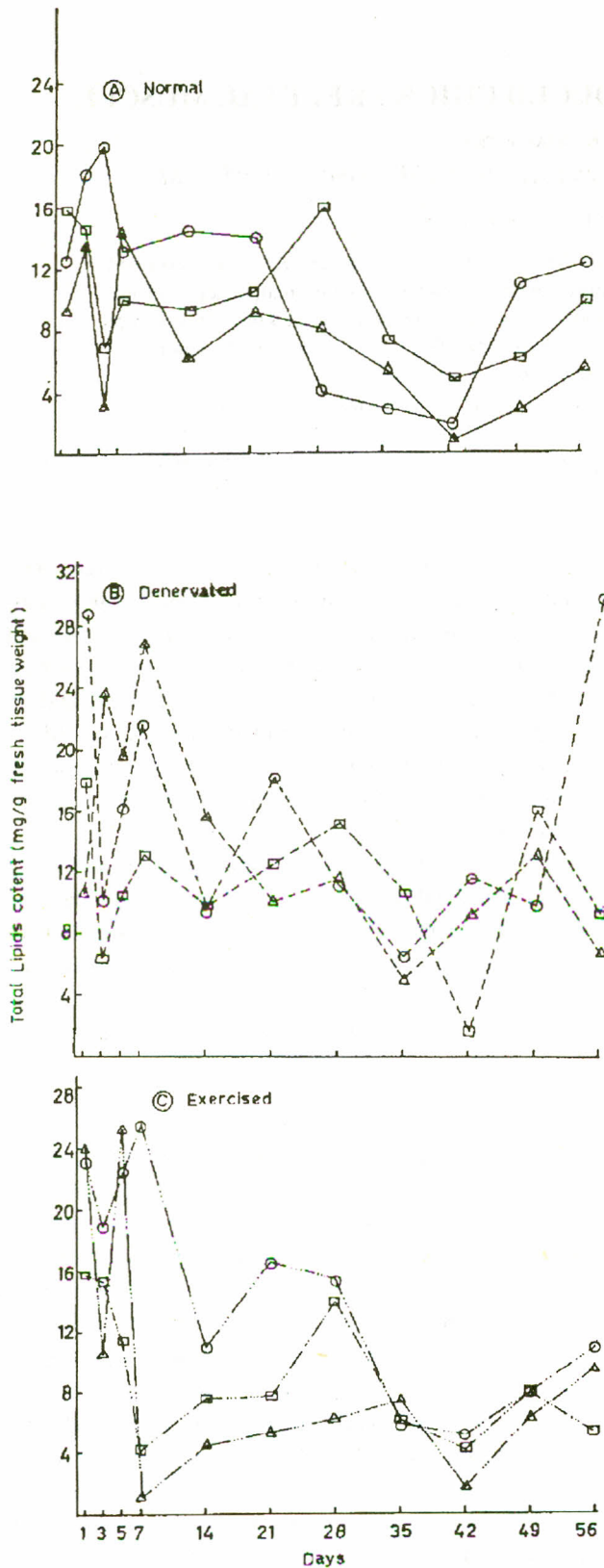


Fig. 1. Total lipid content of 3 chick *Gastrocnemii* under: (A) denervated, (B) exercise, (C) conditions during normal 56 days period of their post hatching growth ( $\Delta$ -*G. lateralis*,  $\square$ -*G. medialis*, O-*G. intermedia*).

extrafibrillar lipids which may be mobilized at the time of need. On the contrary, *G. medialis* which is predominantly red muscle [19, 20], depicts high lipid content throughout its post hatching growth barring transient fall during 42-49 days. Evidently, these lipids are stored within the muscle fibers and are readily utilizable.

Denervation of the muscles results in an overall increase in their lipid levels and such an increase follows a fluctuating pattern during 14-21 days of post-denervation period. The *G. lateralis* shows an immediate response to the ablation of neural control when very steep rise in lipid content is recorded between 3-14 days post denervation. Similar progressive increase in lipid content of muscle has been noticed by various workers [21]. The mechanism of lipid formation and storage is not yet clear but according to some researchers [22] increment in the lipid content is due to rapid utilization of glucose for lipid synthesis. According to these workers lipids so formed are deposited in the muscle fibers and such depositions are observed only in fibres of *G. intermedia*. From the present study, it may be opined that the increment in the lipids may not necessarily be within the fibers but may be deposited in the extrafibrillar region in large quantities. Were it not so, the muscle fibers in all the three *Gastrocnemii* would have exhibited strong histochemical localization (unpublished work). The increase in lipids may also be attributed to their reduced utilization in the denervated muscles.

Under conditions of work-induced stress, large amounts of lipids are recorded during the early stages of investigation but moderate quantities noticed towards the later period. The *G. lateralis* shows an increase in its total lipids ( $24.047 \pm 4.666$ ) compared to the normal muscles which is further enhanced to  $24.309 \pm 3.956$  at 5 days stage. Compared to the *pars lateralis*, the *G. medialis* does not depict any rise in its lipid levels during the early stages of stress. However, between 5-56 days of workoverload, relatively low lipid content is recorded in the muscle barring a transient rise at 28 days stage. The *G. intermedia* behaves much like the *pars lateralis* when it depicts relatively high lipid content between 1-7 days. It is logical to conclude that during the initial stages, the induced exercise stimulates not only the lipogenetic processes but also elevates the rate of mobilisation in the muscles. After 7 days of work stress, not only are these lipids diluted as the result of fiber growth but the lipid mobilization also appears to be retarded thereby resulting in low lipid levels of the muscles after prolonged exercise.

#### References

1. E.L. Kanabrocki, J. Gerent., 18, (1963).
2. E. Turchette, H. Weiss and E. Formingine, Nutr. Dieta., 11, 23 (1969).

TABLE 1. TOTAL LIPID CONCENTRATIONS (mg/g FRESH WEIGHT) IN NORMAL, DENERVATED AND EXERCISED MUSCLES AT DIFFERENT STAGES OF GROWTH  $\pm$  STANDARD ERROR.

Muscles	Period in days										
	1	3	5	7	14	21	28	35	42	49	56
<b>Normal</b>											
<i>G. lateralis</i>	9.285 $\pm$ 1.382	13.575 $\pm$ 2.568	3.162 $\pm$ 0.892	14.446 $\pm$ 1.374	6.200 $\pm$ 0.658	9.212 $\pm$ 1.256	8.151 $\pm$ 1.176	5.396 $\pm$ 0.958	0.962 $\pm$ 0.085	2.822 $\pm$ 0.765	5.507 $\pm$ 0.768
<i>G. medialis</i>	15.895 $\pm$ 2.258	14.636 $\pm$ 2.324	6.912 $\pm$ 0.789	10.008 $\pm$ 0.956	9.333 $\pm$ 0.850	10.483 $\pm$ 2.344	15.996 $\pm$ 2.684	8.425 $\pm$ 1.364	4.935 $\pm$ 0.776	6.102 $\pm$ 0.890	9.892 $\pm$ 0.780
<i>G. intermedia</i>	12.595 $\pm$ 1.358	18.113 $\pm$ 3.560	19.926 $\pm$ 3.439	13.246 $\pm$ 1.836	14.576 $\pm$ 3.560	14.098 $\pm$ 3.408	4.019 $\pm$ 0.668	2.960 $\pm$ 0.508	1.980 $\pm$ 0.678	11.007 $\pm$ 2.536	12.318 $\pm$ 3.058
<b>Denervated</b>											
<i>S. lateralis</i>	10.630 $\pm$ 3.664	23.789 $\pm$ 4.386	19.614 $\pm$ 2.680	26.963 $\pm$ 3.238	15.632 $\pm$ 2.264	10.123 $\pm$ 2.835	11.605 $\pm$ 2.608	5.057 $\pm$ 0.989	9.202 $\pm$ 1.056	13.151 $\pm$ 2.695	6.847 $\pm$ 1.004
<i>G. medialis</i>	17.933 $\pm$ 3.328	6.533 $\pm$ 0.989	10.578 $\pm$ 3.560	13.186 $\pm$ 3.768	9.738 $\pm$ 0.328	12.591 $\pm$ 2.568	15.210 $\pm$ 3.478	10.760 $\pm$ 2.638	1.784 $\pm$ 0.888	16.113 $\pm$ 2.958	9.356 $\pm$ 3.688
<i>G. intermedia</i>	28.969 $\pm$ 4.378	10.276 $\pm$ 1.978	16.320 $\pm$ 2.853	21.733 $\pm$ 3.935	9.526 $\pm$ 1.118	18.237 $\pm$ 2.217	11.241 $\pm$ 1.385	6.520 $\pm$ 0.780	11.769 $\pm$ 0.956	9.971 $\pm$ 0.809	29.923 $\pm$ 4.212
<b>Exercised</b>											
<i>G. lateralis</i>	24.047 $\pm$ 4.666	10.638 $\pm$ 2.854	25.309 $\pm$ 3.956	1.281 $\pm$ 0.098	4.498 $\pm$ 0.353	5.315 $\pm$ 0.468	6.236 $\pm$ 0.738	7.514 $\pm$ 0.954	1.872 $\pm$ 0.065	6.348 $\pm$ 0.515	9.549 $\pm$ 0.962
<i>G. medialis</i>	15.836 $\pm$ 3.435	15.411 $\pm$ 2.689	11.623 $\pm$ 1.324	4.351 $\pm$ 0.357	7.740 $\pm$ 0.542	7.958 $\pm$ 0.648	14.031 $\pm$ 2.048	6.268 $\pm$ 0.998	4.279 $\pm$ 0.899	8.036 $\pm$ 0.948	5.466 $\pm$ 0.348
<i>G. intermedia</i>	23.287 $\pm$ 3.225	18.979 1.998	22.577 $\pm$ 2.532	25.633 $\pm$ 2.842	11.060 $\pm$ 1.424	16.634 $\pm$ 2.058	15.418 $\pm$ 2.218	6.113 $\pm$ 0.368	5.216 $\pm$ 0.242	8.035 $\pm$ 0.538	11.029 $\pm$ 1.612

- D. Kritchevsky, Proceedings 4th European Symposium on Basic Research in Gerontology, Varberg-Sweden (1973).
- K.N. Singh and K. Subbarao, Ind. J. Biochem. Biophys., **16**, 349 (1979).
- S.F. Shvets, P.V. Stapai and I.A. Maker, Dokl. Akad. Nauk. Ukr. SSR. Ser. B. Goel. Khin. Biol. Nauki U, **4**, 77 (1981).
- N. Morisaki, Y. Fujiyama, N. Matsuoka, Y. Saito and A. Kumagai, Gerontology, **30**, 13 (1984).
- A.L. Lehninger, *Biochemistry* (Worth Publishers, Inc. New York, 1975).
- A. White, P. Handler and E.L. Smith, *Principles of Biochemistry* (Blakiston, New York, 1964).
- G.E. Sumnicht and R.A. Sabbadini, Arch. Biochem. Biophys., **215**, 628 (1982).
- E. Wortheimar and V. Benter, Biochem. J., **50**, 573 (1952).
- E. Gutmann, *The Denervated Muscles* (Publishing House of Czechoslovak, Academy of Sciences, Prague, 1962).
- R.L. Dryer, A.R. Tammes, J.E. Routh and W.D. Paul, Proc. Iowa Acad. Sci., **63**, 398 (1956).
- H. Oppenheimer and A.T. Milhorat, Acad. Sci., **94**, 308 (1961).
- H. Wakamatsu, H. Nakamura, K. Ito, W. Anczawa, S. Okajima, S. Okamoto, K. Shigeno and G. Yuichiro, J. Med., **19**, 145 (1970).
- R.K. Malhotra, S. Dhingra and S.S. Katoch, Experientia, **34**, 1206 (1978).
- J. Folch, M. Less and G.H. Slona Stanley, J. Biol. Chem., **226**, 497 (1957).
- S.V. Pandey, R. Parvin and Venkatasubramaniam, Anat. Biochem., **6**, 415 (1963).
- J.C. George and A.J. Berger, *Avian Myology* (Academic Press, New York, 1966).
- S. Morita, R.G. Cassens and E.J. Briskey, Stain Tech., **44**, 283 (1969).
- S. Morita, R.G. Cassens and E.J. Briskey, J. Histochem. Cytochem., **18**, 364 (1970).
- F.L. Humoller, D. Hatch and McIntyre, Am. J. Physiol., **169**, 654 (1952).
- A. Bass and O. Hudlicka, Physiol. Bohemnslov, **9**, 401 (1960).