

EFFECTS OF UNDERNUTRITION AND SEX DIFFERENCE ON SKELETAL MUSCLE FUNCTION IN YOUNG RATS

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(Received on February 17, 1992)

Abstract: The effects of undernutrition and sex difference on skeletal muscle contractile characteristics were studied in young albino rats. The skeletal muscle (gastrocnemius) of 8 week old pups with only one-third normal food intake (undernourished group = UN group; n = 18), as compared to free fed pups (control group = cont. group; n = 16), showed prolongation of contraction time (23.6 ± 1.0 Vs 20.9 ± 0.8 msec, Mean \pm SE, $P < 0.05$) and relaxation time (31.5 ± 1.8 Vs 22.9 ± 1.1 msec, $P < 0.001$) and retention of contraction force and endurance time. In 18 week old rats the effects of sex difference in females (n = 10), as compared to males (n = 10) were prolongation of contraction time in gastrocnemius (32.2 ± 1.5 Vs 27.8 ± 1.5 msec, $P < 0.05$), less force production in gastrocnemius (668.9 ± 48.0 Vs 895.4 ± 93.3 g, $P < 0.05$) and extensor digitorum longus (20.1 ± 3.3 Vs 29.9 ± 2.5 g, $P < 0.05$) and shorter endurance time (160.8 ± 10.2 Vs 187.2 ± 7.1 sec, $P < 0.05$) in soleus. Thus, it is concluded that early undernutrition has prolonged the contraction and relaxation times of the skeletal muscles and the effect of sex difference in the early adulthood was different in different skeletal muscles.

Key words: undernutrition
extensor digitorum longus

gastrocnemius
soleus
isometric contractions

INTRODUCTION

Undernutrition affects the muscle contraction in adults (1,2) and it does much more harm to the body and brain if it occurs during the growth period (3,4) like atrophy of the muscle fibres in skeletal muscle (5). However, detailed reports are not available to show the changes in skeletal muscle contractile characteristics due to undernutrition in young individuals.

The effect of sex difference on various physiological parameters of muscles is well known. Histological studies have shown that women have a higher proportion of type I twitch fibres than men (6). Biochemically, glycolytic enzymes are more in muscles of males than females whereas the potential for oxidative metabolism is higher in females (7,8). Thus, it is likely that sex difference affects the skeletal muscle contractile characteristics also. Guyton, while describing the performances of males and females on the sports field, has pointed out that females show superior performance as compared with that of males in some endurance exercises while males have greater muscle strength than

females (9). The differences in muscle fibres and muscle mass are apparently due to the differences in sex hormone profile which begins at puberty. Thus the extent of sex difference on the contractile characteristics of individual muscles in the early days of adulthood is worth studying and has not received due attention.

Therefore, the present work was designed to study skeletal muscle contractile characteristics in young rats with the objectives of evaluating (i) the changes brought about by early undernutrition and (ii) the early effects of sex difference.

METHODS

Undernutrition experiments : The free fed pups of albino rats, Wistar/NIN in bred (National Institute of Nutrition, Hyderabad, INDIA) on 28th day of age were divided into control group (cont. group, n = 16) and undernourished group (UN group, n = 18), each group containing equal number of males and females. Undernutrition was imposed from 29th day for 4 weeks in UN group by providing food pellets (Goldmohar

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Rat feed, Lipton India Ltd, Bangalore, INDIA) to each pup amounting to one-third of what was consumed by pair fed control pup on each previous day. These pellets contained crude protein 21%, ether extract 5%, nitrogen free extract 53%, crude fibre 4%, vitamins, minerals and trace elements to meet the requirements of rats. Water was given *ad libitum* to both groups of rats. The acute study on skeletal muscle contractions (*in situ*) was performed in a laboratory ($28 \pm 1^\circ\text{C}$) in the following manner at the end of 4 weeks.

In Nembutal anaesthetised (Pentobarbital Sodium; Abbott India Laboratories Ltd, Bombay, INDIA; 40 mg/kg, IP) pup, the tendon of gastrocnemius (GN) was separated from those of soleus and plantaris with minimum possible damage to the blood vessels, and connected to an isometric force transducer with a micrometer adjustment, with the help of 3.0 silk thread. The knee joint was fixed by clamping the needle passing through the distal end of femur and the ankle was also fixed by direct clamping. The sciatic nerve was exposed, the branches for other muscles were severed at the knee joint, and the distal cut end of the nerve was placed on a bipolar stimulating electrode. The exposed muscle was kept warm and moistened with prewarmed mammalian Ringer drops at $37 \pm 1^\circ\text{C}$. The sciatic nerve was stimulated with supra maximal (125% maximal) square wave pulses of 0.2 msec duration by using a stimulator (MS 927 - OS 768 S ECIL, INDIA). While stimulating at 1 Hz, the muscle length was adjusted by the micrometer arrangement to produce maximal isometric contractions which were recorded using the precalibrated force transducer, a DC preamplifier, and a pen recorder (Force transducer : FT 03, DC preamplifier : 7 P1A, pen recorder : Model 7 polygraph; Grass Insts. Co, Quincy, Mass, USA). Calibration of the transducer was done daily, with 40 g hanging. A sensitivity between 25 mm pen deflection/40 g and 2.5 mm pen deflection/40 g was usually used for twitch and tetanic contractions (see below).

Simple muscle twitches: The nerve was stimulated at 1 Hz and 2-3 twitches were recorded at 100 mm/sec paper speed. The maximum twitch force, contraction time (CT) and half relaxation time ($1/2$ RT: time to relax upto 50% from the maximum twitch force) were measured from the polygraph tracings.

Tetanic contractions: The stimulation was delivered for 2 sec at frequencies 10-100 Hz and the interval between the successive frequency stimulations was 2 min. The resulting isometric contractions were recorded at 1 mm/sec paper speed.

Maximum relaxation rate (MRR): The stimulation was delivered at 20 Hz for 2 sec and the responses recorded at 100 mm/sec. The MRR was measured as the percentage decrease in force from the maximum in 10 msec (10). MRR was also measured using 100 Hz.

Endurance time (ET): The stimuli were delivered at 20 Hz and the responses recorded at 1 mm/sec. ET was measured as the time (sec) upto which >50% of the maximum force could be maintained (11). ET was also measured at 100 Hz.

After the study, the animals were sacrificed with over dose of Nembutal.

Sex difference experiments: These experiments were conducted in albino rats (age 18 ± 1 week; $\sigma^7 = 10$, $\phi = 10$ rats) who had free access to the pelleted diet and water. The following protocol was used for studying the contractions in GN, soleus (SOL) and extensor digitorum longus (EDL) *in situ*. First, SOL in the right leg was studied by stimulating the peripheral cut end of the sciatic nerve (without cutting any branches of sciatic nerve) and the contractions were recorded from the tendon of SOL after isolating it from those of GN and plantaris. Then, EDL was studied after a rest period of 30 min by stimulating the same sciatic nerve and recording the contractions from the tendon of EDL connected to the transducer. On the left leg, sciatic nerve was exposed and the branches for SOL and EDL were severed. The tendon of GN was isolated from SOL and plantaris and attached to the transducer for recording the contractions. Simple muscle twitches, tetanic contractions, MRR, and ET were studied in GN, SOL and EDL as described earlier. After the study, the animals were sacrificed with over dose of Nembutal.

Statistical analysis: The data were subjected to statistical analysis using Student's "t" test and 'p' values less than 0.05 were considered to be statistically significant.

RESULTS

(All the values are presented as mean \pm SE).

Effects of undernutrition: The undernourished rats had less body weight as compared to the control rats (56.2 ± 1.4 Vs 122.1 ± 8.7 g). The weight of the GN muscle was also significantly less in UN group (286.0 ± 16.1 Vs 574.3 ± 40.9 mg, $P < 0.001$). But the muscle weight (normalised to 100 g of body weight) was similar in both groups (cont. group : 507.6 ± 25.8 and UN group : 471.4 ± 16.0 mg).

Twitch contractions : In UN group CT was longer as compared to cont. group (23.6 ± 1.0 Vs 20.9 ± 0.8 msec, $P < 0.05$). 1/2 RT was also longer in UN group (31.5 ± 1.8 Vs 22.9 ± 1.1 msec, $P < 0.001$). The twitch force was similar in both groups (UN group : 82.2 ± 12.1 , cont. group : 88.1 ± 20.5 g). But when the twitch force was expressed per gram muscle it was significantly more in UN group (293.7 ± 34.0 Vs 137.7 ± 21.0 g, $P < 0.001$).

Tetanic contractions : The UN group produced similar forces as that of cont. group at all frequencies used in the present study. Interestingly, it was observed that when the forces were expressed per gram muscle the UN group produced significantly greater forces at all frequencies (Fig. 1).

MRR : The UN group showed lower max. relaxation rate as compared to cont. group at 20 Hz (14.6 ± 1.1 Vs $25.3 \pm 2.1\%$, $P < 0.001$). However, at 100 Hz MRR was similar in both the groups (UN group : 18.1 ± 1.9 , cont. group $25.0 \pm 2.8\%$).

ET : Differences in ET were statistically insignificant between the two groups. In UN group ET was 50.8 ± 6.2 and 6.2 ± 0.6 sec at 20 and 100 Hz respectively whereas in cont. group it was 68.6 ± 12.0 and 7.3 ± 0.5 sec at 20 and 100 Hz respectively.

Effects of sex difference : GN in male rats weighed significantly more than in females (893.0 ± 40.5 Vs 731.0 ± 46.2 mg, $P < 0.02$). However, SOL and EDL weighed similar in both sexes (SOL σ^7 : 83.2 ± 4.7 , SOL ϕ : 72.9 ± 6.6 , EDL σ^7 : 69.5 ± 4.9 , EDL ϕ : 55.7 ± 4.8 mg). When normalised for 100 g of body weight, the weight of GN was 543.3 ± 29.4 and 538.8 ± 23.4 , that of SOL was 49.7 ± 2.4 and 51.1 ± 2.9 and that of EDL was 41.9 ± 2.3 and 40.3 ± 8.1 mg in males and females respectively. There was no sex difference in these normalised muscle weights for any given muscle.

Twitch contractions : The peak force produced by EDL was significantly less in female rats than male rats (20.1 ± 3.3 Vs 29.9 ± 2.5 g, $P < 0.05$). CT of GN was significantly longer in female rats than male rats

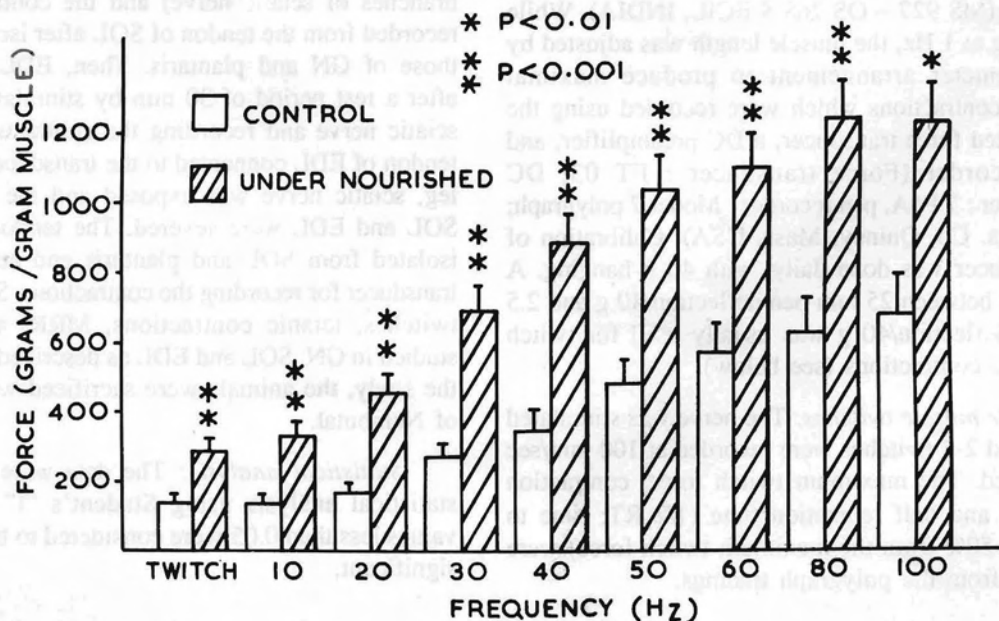


Fig. 1 : Frequency force diagram of gastrocnemius muscle in con. group and UN group.

(32.2 ± 1.5 Vs 27.8 ± 1.5 msec, $P < 0.05$). The other contractile parameters were similar in both sexes (Table I).

Tetanic contractions : The force produced by GN was less in females than males at all frequencies of stimulation but the differences were significant ($P < 0.05$) only at 50, 60 and 80 Hz. The forces developed by EDL and SOL were similar in both sexes (Table II).

TABLE I: Effects of sex difference on Contraction time (CT), half relaxation time (1/2 RT), Maximum relaxation rate (MRR), Endurance time (ET) in male and female rats. (Values are mean \pm SE).

| Muscle | Sex | CT (msec) | 1/2 RT (msec) | Peak twitch force (g) | MRR % | | ET (sec) | |
|---------------------------|--------|-----------------|------------------|-----------------------------|----------------|----------------|-------------------|----------------|
| | | | | | 20 Hz | 100 Hz | 20 Hz | 100 Hz |
| Gastrocnemius | Male | 27.8 \pm 1.5 | 32.2 \pm 2.7 | 198.3 \pm 13.6 | 19.0 \pm 1.7 | 19.4 \pm 1.1 | 59.0 \pm 5.4 | 6.2 \pm 0.4 |
| | Female | 32.2 \pm 1.5* | 33.3 \pm 2.6 | 166.8 \pm 23.0 | 19.0 \pm 1.5 | 17.4 \pm 1.3 | 76.2 \pm 8.7 | 6.2 \pm 0.5 |
| Soleus | Male | 56.5 \pm 2.4 | 91.5 \pm 4.2 | 24.1 \pm 1.9 | 5.3 \pm 0.4 | 5.8 \pm 0.4 | 187.2 \pm 7.1 | 26.5 \pm 3.3 |
| | Female | 57.5 \pm 4.0 | 91.5 \pm 12.5 | 24.4 \pm 2.3 | 6.6 \pm 0.7 | 6.8 \pm 0.8 | 160.8 \pm 10.2* | 24.9 \pm 2.7 |
| Extensor digitorum longus | Male | 27.0 \pm 2.3 | 36.0 \pm 2.2 | 29.9 \pm 2.5 | 20.2 \pm 2.3 | 18.3 \pm 1.9 | 42.3 \pm 1.4 | 6.9 \pm 0.5 |
| | Female | 23.0 \pm 1.5 | 34.0 \pm 0.6 | 20.1 \pm 3.3* | 17.0 \pm 1.3 | 15.4 \pm 1.7 | 46.7 \pm 4.8 | 6.6 \pm 0.3 |

* $P < 0.05$

TABLE II: Effects of sex difference on Tetanic forces (g) in male and female rats (Values are mean \pm SE).

| Muscle | Sex | 10Hz | 20Hz | 30Hz | 40Hz | 50Hz | 60Hz | 80Hz | 100 Hz |
|---------------------------|--------|------------------|------------------|------------------|------------------|-------------------|-------------------|-------------------|------------------|
| Gastrocnemius | Male | 192.1 \pm 14.7 | 319.1 \pm 51.7 | 462.7 \pm 53.8 | 627.3 \pm 56.7 | 729.3 \pm 50.2 | 779.0 \pm 60.6 | 895.4 \pm 93.3 | 822.5 \pm 86.6 |
| | Female | 177.5 \pm 22.8 | 238.5 \pm 29.4 | 378.4 \pm 60.9 | 497.1 \pm 69.0 | 558.3 \pm 51.6* | 598.3 \pm 41.0* | 668.9 \pm 48.0* | 685.5 \pm 48.7 |
| Soleus | Male | 60.5 \pm 5.8 | 78.6 \pm 6.3 | 90.3 \pm 6.1 | 98.2 \pm 6.6 | 106.7 \pm 7.2 | 111.2 \pm 6.7 | 112.1 \pm 6.8 | 112.9 \pm 7.5 |
| | Female | 70.4 \pm 7.2 | 90.9 \pm 7.1 | 101.1 \pm 8.2 | 107.8 \pm 9.2 | 110.4 \pm 9.0 | 113.4 \pm 8.9 | 117.0 \pm 8.9 | 117.8 \pm 8.8 |
| Extensor digitorum longus | Male | 33.1 \pm 4.9 | 49.5 \pm 7.7 | 79.0 \pm 9.9 | 98.0 \pm 12.2 | 106.3 \pm 12.2 | 109.2 \pm 13.1 | 117.2 \pm 15.6 | 119.6 \pm 18.3 |
| | Female | 25.2 \pm 5.1 | 36.9 \pm 7.7 | 50.0 \pm 9.8 | 70.1 \pm 13.2 | 78.6 \pm 13.8 | 86.9 \pm 14.0 | 94.7 \pm 13.7 | 95.8 \pm 12.7 |

* $P < 0.05$

MRR : There was no sex difference in MRR at both stimulation frequencies in any of the muscles (Table I) studied.

ET : ET of SOL was significantly shorter in females as compared to males (160.8 ± 10.2 Vs 187.2 ± 7.1 sec, $P < 0.05$) at 20 Hz. No other significant differences could be observed in ET (Table I).

DISCUSSION

Undernutrition and muscle contraction : The skeletal muscle of undernourished rats has shown preservation of the contraction force and endurance time with prolongation of contraction and relaxation times. Similar changes have been reported in earlier studies with different forms of undernutrition (2,12) and partly

explained on the basis of elevated intracellular calcium (2). As there was no shift to left or shift to right (11) the differential atrophy of muscle fibres is unlikely in the present study. The preservation of contraction force in UN group muscles and even the greater force when corrected for the muscle weight, might be due to the preservation of contractile components with the reduction in noncontractile components. Kelsen et al

in the study with 33% feeding for 4 weeks, have also reported that the mechanical efficiency of the myofibrillar material was not affected by undernutrition (13). These probable changes viz, atrophy of non contractile components with the preservation of contractile components, may also explain the lack of differences in the tetanic contraction, force-frequency relationship and endurance time.

The observations made during the present study (unpublished) that the contractions of gastrocnemius muscle of the undernourished group due to the so called "direct stimulation" and the sciatic nerve stimulation were similar, do not favour the possibility that the nerve and the neuromuscular junction have been affected in the undernourished muscles.

Sex difference and muscle contraction : The skeletal muscle contraction in the present study showed a few sex differences. The significant differences in forces shown by EDL and GN were not there when the forces were expressed per gram muscle, indicating that

these differences were due to the differences in the muscle weights. The longer CT in GN of females might be due to the observation that the skeletal muscles of females have greater proportion of slow fibres than males (6). The shorter ET of SOL in females at 20 Hz might be due to the greater rate of force production. At 20 Hz, SOL in females produced significantly higher force in one second (1281.0 ± 85.4 Vs 962.9 ± 82.5 g per g muscle, $P < 0.02$). Komi et al have shown that adult females have lesser muscle force, muscle power and velocity of contraction than adult males (14) and these effects of sex difference seem to begin occurring in the early days of adulthood as evidenced by a few differences between the sexes in the present data.

Thus, the present study shows that food restriction in the early age results in prolongation of contraction and relaxation times of the skeletal muscle. The early manifestations of sex difference in female rats were longer contraction time of GN, less force production of GN and EDL and shorter endurance time in SOL.

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