THE EFFECTS OF STATIC STRETCHING AFTER STRENUOUS TRAINING ON ULTRASTRUCTURE AND FLEXIBILITY OF RATS' GASTROCNEMIUS

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The purpose of the present study was to investigate effects of static stretching after strenuous training on the ultrastructure and flexibility of rats' gastrocnemius. 24 male Sprague-Dawley rats were randomly divided into three groups: normal control (NC), training control(TC) and stretching group(ST). The results were as follows: 1) The myofilaments became supercontracted and Z discs were obscure in TC. On the contrary, the myofilaments arranged orderly and the Z lines were clear and the mitochondrial cristas were manifolded in ST. 2) Compared with NC, the ultimate tensile strength of gastrocnemius was increased in TC, while the Max. deformation of gastorcnemius was decreased. However, the Max. deformation in ST was increased than that of NC. The conclusion was that the ultrastructure of muscle was resumed and the ability of distortion and flexibility was improved by static stretching, which decreased the risk of injury.

KEY WORDS: myofilaments, skeletal muscle, biomechanics, the Max. deformation

INTRODUCTION: Stretching is widely performed before and after training and exercising, including static stretching and dynamic stretching. Stretching is important for reducing injury and improving performance in sports and for physical fitness. Athletes are often given stretching protocols to improve their flexibility, decrease stiffness and delay the onset of muscle soreness, decrease the risk of injury, and enhance athletic performance. Active warm-up may be protective against muscle strain injury. On the contrary, the controversial views about stretching were suggested by some researchers. Rob & Michael (2002) reported that stretching before or after exercising did not confer protection from muscle soreness. Stretching before exercising did not seem to confer a practically useful reduction in the risk of injury (Larsen et al. 2005). A static stretch regimen had no effect on knee joint position sense in healthy volunteers. Taken together, these previous results might result from different research with different training methods and exercise intensity. The research on the effects of stretching on muscle function was insufficient. Although results from single stretch models have been informative about the causal factors in muscle injury, no attempt has been made to investigate the influence of static stretching on the ultrastructure and biomechanics parameters after strenuous exercising for a long period in vivo. We hypothesized that stretching might alter the ultrastructure of skeleton muscle, which in turn affected the flexibility and the force of muscle. The purpose of the present study was to investigate the effects of static stretching after strenuous exercising on structure and function of rats' gastrocnemius for a long period, and provide evidence for training and exercising.

METHODS:

Animals and treatments: In order to eliminate the existence of any sex-related differences in response to exercise, the male Sprague-Dawley rats were selected in all experiments. The animal Care and Use Committee of Hebei Science and Technical Bureau in PRC approved the experimental protocol, which was adherence to the guidelines of the Care and Use of Laboratory Animal published by the U.S. Department of Health and Human Services. 24 rats (130~150 g, 6-week old, bought from Hebei Medical University) were randomly divided into three groups: normal control group (NC, remained sedentary), training control group (TC, running on treadmill), and stretching group (ST, static stretching following training). TC performed treadmill training for 5 weeks (5days/week, 20min/day). The target speed in each week was 15m/min, 22 m/min, 27 m/min, 31 m/min, 35 m/min (Zhao Z.Y., Feng L.S., & Zong P.F. 2000). ST performed static stretching after treadmill training. The static stretching consisted of a 60 second stretch followed by a 60 second pause, repeated three times (Bandy W.D., Irion J.M., &Briggler M. 1998). Rats were on their backs, the joint position of the animal between coxa, knee and anklebones remained 180^0. Static stretching was performed by changing the angle between the tibia and the plantar surface of the foot flexed from 180^0 to 90^0. Rats were housed in stainless steel rust-free cages at 22℃ - 24℃, the relative humidity was 45%~55%. The rats were analyzed after 5 weeks running and were sacrificed 36h after the last exercise.

Assessment of ultrastructure of gastrocnemius by transmission electron microscopy: Rats were anesthetized with 0.4% pentobarbital sodium (1 ml/100 g). After perfusion with ice-cold phosphate-buffered saline (pH7.4) through the left ventricle, the gastrocnemius of each rat was removed and quadrate-shaped sections 1.0 mm^3 in size were cut down at 4℃. The specimens were fixed in 4% glutaraldehyde for 2 hour and post-fixed in 1% osmic acid for 1 hour at 4℃. After dehydration with a graded series of acetone concentrations, the samples were washed twice for 15 min in 100% dry acetone, and then were embedded in epon resin. Ultrathin sections (50 nm) were stained with lead citrate and uranyl acetate and then myofilaments of the gastrocnemius were observed under Hitachi 7000 (Tokyo, Japan) transmission electron microscope (TEM) at a magnification of 10 000x.

Assessment of the area of cross section of gastorcnemius: Rats were anesthetized with 0.4% pentobarbital sodium (1 ml/100 g). After perfusion with 4% ice-cold phosphate-buffered formaldehyde (pH7.4) through the left ventricle, the gastrocnemius of each rat was removed. Following fixation in Bouin solution, tissue was placed in 50% ethanol, dehydrated in ascending ethanol, and embedded in paraffin. Sections of 6µm were sliced up by automatism slicer (LEICA RM2155) and then mounted onto albumen glycerin-coated slides. After deparaffinization in xylene and rehydration through graded ethanol, sections were stained in hematoxylin and eosin, and then dehydrated in ascending ethanol. The area of cross section of gastorcnemius was accounted by Imagine Analysis System under light microscope (Olympus BX50).

Quantification of flexibility with biomechanics testing: After anesthetized with 0.4% pentobarbital sodium, the skin was removed and gastorcnemius was dissected from leg and measured the length and perimeter (1 cm form the adhesion site). The dissection was carried out in an air-conditioned room and evaporation from the muscles was compensated by towels dampened with physiological saline. Gastrocnemius stretch experiment was performed on Universal Testing Instruments (SHIMADZU, Japan), according to the manufacture's instructions. Thighbone and shinbone of specimens were fixed up on the clamps respectively. The specimens were snapped by 500 mm/min load velocity. According to the matching Test Software, we got the strain-distortion curve, and the ultimate tensile strength, the maximum deformation ability and the elastic rigidity of gastrocnemius were calculated.

Data analysis: In order to avoid bias, the identities of experimental groups were assigned as numbers, and not revealed for the lab assistants. The results were expressed as means ± SD. Statistical analysis was performed using One-Way ANOVA of Statistical Product and Service Solutions (SPSS11.0) followed by a Student-Newman-Keuls for multiple comparisons. All main effects and interaction terms were considered significant when p values were less than 0.05.

RESULTS:

Effects of static stretching on ultrastructure of rats' gastrocnemius: Histological and electron microscopic analyses were carried out on gastrocnemius samples of rats. The area of the cross section of gastorcnemius was increased after 5-week treadmill training ($P<0.01$), however, the cross section of gastorcnemius in ST was smaller than that of TC ($P < 0.05$) (Table 1). Under the transmission electron microscope, myofilaments were in order and A bands and I bands were regular, and the structure of mitochondria was clear in NC. The situation was different in TC after 5-week treadmill training. The myofilaments became supercontracted and fouled up. The M lines and the Z lines were contorted now and then and the spacing got narrow. Some of the Z discs were obscure, showing z disc streaming. The mitochondrias were swollen and some of the mitochondrial cristas were broken. Dissolution and slight edema of individual crista were observed. Some of the mitochondrial membrane was fractured. While the myofilaments arranged orderly and the Z lines were clear in ST. The number of mitochondrias was increased and some of the mitochondrial cristas were manifolded.

Table1 Effects of static stretching on the biomechanics parameters of rats' gastrocnemius.

group	cross section of gastrocnemius (μm^2)	Ultimate tensile strength (N/mm^2)	Max. deformation (mm)	Elastic rigidity (N/mm)
NC	1318.04±63.09	0.30±0.05	18.30±0.65	1.58±0.21
TC	1909.28±226.40▲▲	0.43±0.08▲	15.75±1.39▲▲	3.36±0.46▲▲
ST	1672.55±157.33△△*	0.50±0.02△	21.13±0.78△△**	2.81±0.17△△*

Note: Values are means ± SD, n = 8. Compared between TC and NC ▲ $p < .05$, ▲▲ $p < 0.01$; Compared between ST and NC △ $p < .05$; △△ $p < .01$; Compared between TC and ST * $p < .05$, ** $p < .01$.

Compared with NC, the ultimate tensile strength of gastrocnemius was improved in ST and TC after treadmill training ($p < .05$). The Max. deformation of gastorcnemius in TC was decreased compared to NC ($p < .01$). On the contrary, the Max. deformation in ST was increased than that of NC and TC ($p < .01$). The elastic rigidity of gastrocnemius was enhanced in TC and ST ($p < .01$), and the elastic rigidity in TC was higher than that of ST ($p < .05$) (Table 1).

DISCUSSION: Strenuous training is performed by athletes in order to enhance performance. However, it is well established that prolonged exhaustive endurance exercise could induce skeletal muscle damage and temporary impairment of muscle function. Although skeletal muscle has a remarkable capacity for repair and adaptation, the strenuous exercise ultimately resulting in an accumulation of chronic skeletal muscle pathology, such as onset of muscle soreness, risk of injury. In the present study, after 5-week strenuous exercising, the area of the cross section of gastrocnemius in ST and TC were increased compared with NC. The strength of muscle is relative to the area of the cross section, but if the area is too big, the achievement of sports would be decreased, and the risk of injury will be increased. The cross section of gastorcnemius in ST was smaller than that of TC, which will be helpful to decrease the risk of injury. The myofilaments became supercontracted and the M lines and the Z lines were contorted and obscure. The mitochondrias were swollen and some of the mitochondrial cristas were broken in TC. Although the ultimate tensile strength and the elastic rigidity of gastrocnemius in TC were increased, the Max. deformation was decreased, which showed the decreased flexibility and enhanced the risk of injury. The flexibility is relative to the achievement of sports and a key component for injury prevention and rehabilitation. Some coaches and athletes thought much of the training of strength, but did little on the training of flexibility. The training of flexibility may make the effects of the training of strength better.

Static stretching has also been recommended to prevent injury and improve performance by increasing flexibility (Taylor et al.1990). The data indicated that static stretching after strenuous training had changed the ultrastructure of gastrocnemius. In ST group, the myofilaments arranged orderly and the Z lines were clear. The number of mitochondrias was increased and some of the mitochondrial cristas were manifolded. The altered structure improved the function of muscle. The Max. deformation of the gastorcnemius in ST was increased than that of TC, the elastic rigidity was decreased compared with TC, which promoted flexibility of gastrocnemius. Decreased stiffness may be associated with the little risk of injury and muscle soreness.

The mechanism of the decreased stiffness and enhanced flexibility after stretching is unknown. A conceivable mechanism for the effects of static stretching after exercising proposed by Vaittinen (1999). Desmin is the bridge of Z discs. After exercising, the number of losing desmin is increased, which results in the destroied Z discs. The expression of desmin could be enhanced after strenching, which would resume Z discs. In the present

study the myofilaments and Z lines in TC were contorted, but those in ST were in order. Static stretching after treadmill training may help the muscle recover.
It has been documented that the potential mechanism for reduced risk of injury with increasing flexibility was the change in the viscoelastic properties of muscle-tendon units (Willson et al. 1991; Grobler et al. 2004). Their study showed it was possible to quantify the viscoelastic properties (stiffness and hysteresis) of human tendon structures in vivo using ultrasonography. Furthermore, static stretching decreased the viscosity of tendon structures as well as increased the elasticity (McNair et al. 2004). This provides a physiological background for reducing passive resistance and improving joint range of motion after stretching.
Previous studies have suggested that after training, the cerebra remain the state of exciting, which excites αmotor nerves and γmotor nerves. The muscle spindle remain the state of exciting. Then the muscle will remain the state of convulsion. The tendon will be exciting with the static stretching. Then theαmotor nerves will be restrained. The state of convulsion of the muscle will be weakened.
In conclusion, the study provided the first evidence for the changes of ultrastructure of gastrocnemius and the improved flexibility by static stretching after strenuous training. Our current understanding of static stretching-mediated regulation of flexibility is still incomplete. Further studies are necessary to characterize the regulatory mechanism of static stretching.

CONCLUSION: The static stretching following training could decrease the risk of injury by resuming the ultrastructure of rats' gastrocnemius, and improving the ability of distortion and flexibility.

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