Biochemical and Histological Study on the effect of muscular exercise on skeletal muscle of adult albino rats

Wael Mohamed Elmashad¹ and Naglaa Ibrahim Sarhan²

Physiology ¹, Histology² Departments, Faculty of Medicine, Tanta University, Egypt. 
( waeelmarshad@yahoo.com, nsarhan2006@hotmail.com ) nahelekmashed@gmail.com

Abstract: Background: Health benefits of regular physical exercise are well known. Exercise induced muscle damage (EIMD) especially of unaccustomed exercise or that contains high frequencies of eccentric muscle actions, is well documented. Low physiological levels of reactive oxygen species (ROS) are generated in the muscles to maintain the normal tone and contractility, but excessive generation of ROS promotes contractile dysfunction and induces tissue damage. Aim of the work: This work was carried out to study the effect of muscular exercise for 15 minutes on the serum sodium and potassium level and also on the histological structure of skeletal muscles of Adult Albino rats. Material & methods: This study was carried on 25 adult albino rats divided into two groups, the first one served as control “Group A” (5 rats) and the second (20 rats) “Group B” exercised for 15 minutes; at the end of the experiment the animals were sacrificed just after exercise (subgroup B1) and 60 minutes later for (subgroup BII). Under anesthesia the lower limbs were dissected to obtain Gasterconemius muscle and prepared for biochemical & physiological study and histological study. Results: Biochemical analysis showed highly statistically significant difference between control and run groups for both Na⁺ and K⁺ serum level. Hypernatremia and hypokalemia were observed in subgroup B1 in association with prominent histological alterations in the form of splitting, disorganization, fragmentation of the myofibrils and mitochondrial disruption. All these changes were ameliorated in subgroup BII but did not reach the control level. Conclusion From the present study it could be concluded that muscular exercise induced significant hyponatremia and Hyperkalemia in association with prominent histological alterations in skeletal muscles immediately after exercise. All these changes were ameliorated partially in subgroup BII.

Key word: Exercise, skeletal muscle, Na⁺ and K

1. Introduction

Exercise and mechanical loading have been shown to be beneficial to the skeleton, as evidenced by numerous studies in animals (1-4). Skeletal muscle cramps are a common affliction in sports and numerous other physical activities. Even highly fit athletes must sometimes succumb to debilitating cramping episodes and some often compete with concern, knowing that these painful, involuntary muscle contractions can appear seemingly without warning or apparent cause. Skeletal muscle overload and fatigue from overuse or insufficient conditioning can prompt muscle cramping locally in the overworked muscle fibers (5-7). During sports competition and training or a variety of other intense physical activities, repeated or extended loading on selected muscles can lead to muscle or tendon strain and local fatigue. The muscle fatigue hypothesis suggests that such a scenario can prompt an excitatory alteration (increase) in muscle spindle afferent activity and a concomitant decrease in Golgi tendon organ inhibition leading to abnormal motor neuron control and sustained motor neuron activity (8,9). It has been speculated that these muscle symptoms occur as a result of a breakdown in electrolyte balance due to a loss of sodium and chloride (Na⁺ Cl⁻) during excessive sweating and a possible reduced muscular cell membrane potential due to a decreased serum calcium ion concentration (10). During a single long race, match, game, or training session or consequent to multiple same- or repeated-day exercise bouts, a sizeable whole-body exchangeable sodium deficit develops when sweat sodium and chloride losses measurably exceed salt intake (11, 12). The estimated sweat-induced loss of 20% to 30% of the exchangeable Na⁺ pool has been noted with severe muscle cramping (13). How readily this occurs depends upon sweating rate (14), sweat sodium concentration (typically 20-80 mmolL⁻¹) (12, 15), and dietary intake (16). To compensate for the loss in plasma volume during exercise, prompted in part by extensive sweating, water from the interstitial fluid compartment shifts to the intravascular space (17, 18). As sweating continues, the interstitial fluid compartment becomes increasingly contracted (17). This can persist even after exercise, as sweating continues and body temperature returns to a pre-exercise level (19).

2. Material and Methods
The present study was carried out on 25 adult albino rats, weighing from 150 to 200 gm. Male or female Wistar rats were selected for the study at 4 weeks of age and randomly divided into control and running groups. They were housed in clean properly ventilated cages under the same environmental conditions with free access to food and water throughout the whole period of the experiment. The animals were divided into two groups:

Group “A” (Control group): Five rats remained sedentary and served as controls.

Group “B” (Run Group): included 20 animals run on a treadmill for 15 minutes subdivided to 2 equal subgroups:

1) Group BI: included 10 animals sacrificed just after exercise.

2) Group BII: included 10 animals sacrificed 60 minutes rest after exercise.

Biochemical and physiological study (measurements of Na+ K+)

Just after running, the rats were sacrificed and blood samples were obtained from (retroorbital plexus or cardiac puncture for measurement of serum levels of Na+ and K+ using the commercially available kits (20, 21).

At the end of the experiment, the animals were scarified under anesthesia and the lower limbs were dissected to obtain Gasterconemius muscle for light and electron microscopic examination. Histological study:

a- Light microscopic study; specimens of the muscles were fixed in 10% formalin for preparation of paraffin blocks. Five μm sections were cut and stained by Hematoxylin and Eosin (17).

b- Electron microscopic study; the muscle specimens were immediately fixed in 2.5% phosphate buffered gluteraldehyde (pH 7.4) at 4°C for 24 hours and post fixed in 1% osmium tetraoxide for one hour, then dehydrated in ascending grades of ethanol. After immersion in propylene oxide, the specimens were embedded in epoxy resin mixture. Semithin sections (1μm) were cut, stained with toluidine blue and examined by light microscopy to choose the best area for ultrathin sectioning. Ultrathin sections (80-90nm) were stained with uranyl acetate and lead citrate and were examined and photographed with a JEOL transmission electron microscopy in Faculty of Medicine Tanta University (19).

Statistical analysis:

One way ANOVA is used to test significant differences between groups and to get Mean and Standard Deviation (SD) for each group with 95% Confidence Interval (CI) for Mean. Using Post Hoc Tests comparing difference between each two group’s level of significance for group BI and BII over control Group A. The mean difference is significant at the 0.05 level.

3. Results

I-Physiological Results

Using one way ANOVA, it showed highly statistically significant difference between control group A and running group B for both Na+ and K+ with P value <0.001 (Table 1)

Post Hoc Tests showed highly statistically significant difference in Group BI and BII in low sodium level when compared with control group A (T - test was 21.093 and 4.062 respectively with P value <0.001). There was also highly statistically significant low sodium level for Group BI over Group BII (T-test was -19.63 with P value <0.001) (Diagram1).

As regard Potassium level, it revealed statistically significant increase in Group BI in comparison to control Group A (Diagram 2), (T-test was -8.250 with P value <0.001). But no difference was observed between Group BII and control Group A (t –test is -0.39 and P value >0.05). Also there was highly statistically significant increase in potassium level in Group BI compared to Group BII, (T-test is -8.125 with P value <0.001) (Table 2).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Group</th>
<th>Run Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A (n=5)</td>
<td>Group BI (n=10)</td>
</tr>
<tr>
<td>Na+ (mmol/L) Mean +SD</td>
<td>141.4±3.0060</td>
<td>116.020 ± 2.3351</td>
</tr>
<tr>
<td>Minimum value</td>
<td>136.3</td>
<td>112.3</td>
</tr>
<tr>
<td>Maximum value</td>
<td>145.2</td>
<td>120</td>
</tr>
<tr>
<td>CI 95%</td>
<td>139.260-143.560</td>
<td>114.350-117.690</td>
</tr>
<tr>
<td>P Value</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>K+ (mmol/L) Mean +SD</td>
<td>10.6±.8634</td>
<td>12.780±.6179</td>
</tr>
<tr>
<td>Minimum value</td>
<td>8.7</td>
<td>11.7</td>
</tr>
<tr>
<td>Maximum value</td>
<td>11.5</td>
<td>13.6</td>
</tr>
<tr>
<td>P Value</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>
Table (2): Comparison (Post Hoc Test) between different studied groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Compared Group</th>
<th>Compared Group</th>
<th>P value</th>
<th>T -test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Na+ (mmol/L)</td>
<td>Control Group A</td>
<td>Group BI</td>
<td>&lt;0.001</td>
<td>21.093</td>
</tr>
<tr>
<td></td>
<td>Group BI</td>
<td>Group BII</td>
<td>&lt;0.001</td>
<td>4.062</td>
</tr>
<tr>
<td></td>
<td>Group BI</td>
<td>Group BII</td>
<td>&lt;0.001</td>
<td>-19.63</td>
</tr>
<tr>
<td>Potassium K+ (mmol/L)</td>
<td>Control Group A</td>
<td>Group BI</td>
<td>&lt;0.001</td>
<td>-8.250</td>
</tr>
<tr>
<td></td>
<td>Group BII</td>
<td>&gt;0.05</td>
<td>-0.39</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Group BI</td>
<td>Group BII</td>
<td>&lt;0.001</td>
<td>8.125</td>
</tr>
</tbody>
</table>

The running group (Subgroup BII) examined after 60 minutes rest revealed disappearance of most the histological changes and mild focal splitting of the myofibers in association with focal mononuclear cellular infiltration were the only observed findings (Fig. 3).

Diagram 1: Mean Sodium (Na+) Level in control group A, Group BI, BII.

Diagram 2: Mean Potassium (K+) Level in control group A, Group BI, BII.

Histological results:

Light microscopic examination of Hematoxylin and eosin stained sections of the skeletal muscle fibers of control (Group A) revealed acidophilic parallel cylindrical striated peripherally multinucleated fibers in their longitudinal sections (Fig. 1).

The running group (Subgroup BI) exercised for 15 minutes revealed more pronounced histological changes in the form of wide splitting of the skeletal muscle fibers in association with fibrillolysis and nuclear internalization (Fig. 2).

Fig(1): A photomicrograph of a longitudinal section in the skeletal muscle of an adult control albino rat showing elongated unbranched muscle fibers (star) with acidophilic cytoplasm and striated appearance. Their nuclei are flat and peripherally located (arrow). (H&E 400)

Fig(2): A photomicrograph of a longitudinal section in the skeletal muscle of subgroup BI exercised for 15 minutes showing wide splitting of the muscle fibers (star) and fragmentation of muscle fibers (►) together with nuclear internalization (→) (H&E 400)
Fig(3): A photomicrograph of a longitudinal section in the skeletal muscle of subgroup BI exercised for 15 minutes then 60 minutes rest showing mild splitting (→) and localized mononuclear cellular infiltration (►). (H&E X200).

The electron microscopic examination of the ultra thin sections of gastrocnemius muscle of the control group revealed normal structure of skeletal muscle. They displayed sub sarcolemmal oval nuclei with finely dispersed chromatin except those of chromatin islands and peripheral chromatin near the nuclear membrane (Fig. 4). The myofibrils were arranged in sarcomeres between Z-lines with the light I-band in the periphery and dark A-band in the middle. Light H-zone bisected by dark M-line could also be seen in the centre of the dark A-band. Between the myofibrils the sarcoplasmic reticulum and mitochondria were seen. Mitochondria were uniformly distributed in rows separating the myofibrils (Fig.5).

Fig. (4): An electron micrograph of gastrocnemius muscle of control group (Group A) showing subsarcolemmal nucleus (N) with peripherally condensed chromatin (x 3000)

Fig. (5): An electron micrograph of ultrathin section of the gastrocnemius muscle of control group (Group A) showing the normal banding pattern of regularly alternating I band (I) which is bisected by Z lines and A band (A) which is bisected with H zone. Notice M line in the middle of H zone (wavy arrow), mitochondria (→) and glycogen (►) granules in the regular spaces between the myofibrils (x 3000).

Fig. (6): An electron micrograph of ultrathin section of the skeletal muscle of (subgroup BI) showing disorganization, fragmentation as well as separation and focal lyses of the sarcomere elements (→). The mitochondria were disrupted at these sites (►). Notice also absence of mitochondrial X4000

The electron microscopic examination of the ultra thin sections gastrocnemius muscle of the (subgroup BI) displayed disturbance loss of the architecture in the form of disorganization, fragmentation as well as separation and focal lyses of the sarcomere elements. The mitochondria were disrupted or showed subsarcolemmal accumulation or complete absence from the examined fields (Figs.6 & 7).

On the other hand subgroup BII showed displayed histological picture nearly similar to the control group (Fig.8).
Fig. (7): An electron micrograph of ultrathin section of the skeletal muscle of (subgroup BI) showing subsarcolemmal accumulation of the mitochondria (►). X2500

Fig. (8): An electron micrograph of ultrathin section of the skeletal muscle of (subgroup BII) showing regular arrangement of the myofibrils in sarcomeres between Z lines (Z) with .

4. Discussion

The ability of mitochondria to interact and fuse in skeletal muscle fibers is a relatively discovered property, and whether these processes are altered by exercise is unknown. To isolate the acute effect of exercise on mitochondrial dynamics, we applied a “non stressful” voluntary running paradigm in rats, following their normal physical activity pattern, and examine mitochondrial morphology on electron micrographs from the longitudinal and cross-sectional axes of skeletal muscle.

We studied 20 animals divided into two equal subgroups exposed for running on a treadmill for 15 minutes killed just after exercise (subgroup BI) and after 60 minutes (subgroup BII). After dissection, gastrocnemius muscle physiological and histological studies had done.

Light microscopy of gastrocnemius muscle of (subgroup BI) revealed mild focal splitting of the myofibers in association with focal mononuclear cellular infiltration while (subgroup BII) displayed histological picture nearly similar to the control group.

All these morphological alterations were supposed to be secondary to oxidative stress induced by muscular exercise. Mitochondria have generally been cited as the predominant source of reactive oxygen species (ROS) in muscle cells (22). It was recorded formerly that contracting skeletal muscles produce free radicals and excessive generation of ROS promotes contractile dysfunction resulting in muscle weakness and tissue damage (23, 24).

In skeletal muscle, each action potential is elicited by a Na\(^+\) influx and K\(^+\) efflux. These passive fluxes are counteracted by the Na\(^+\)–K\(^+\)-ATPase (Na\(^+\)–K\(^+\) pump), which actively transports Na\(^+\) back out of and K\(^+\) back into the cell, allowing maintenance of the transmembrane Na\(^+\) and K\(^+\) gradients. However, during periods of frequent action potentials, the accelerated passive ion fluxes exceed the activity of the Na\(^+\)–K\(^+\) pumps, resulting in a rundown of the sarcolemmal Na\(^+\) and K\(^+\) concentration gradients and loss of membrane excitability (25).

Some studies have found increases in plasma [K\(^+\)] from ~4 to 12 mM and reductions in plasma [Na\(^+\)] from ~140 to ~130 mM (26,27). Although the exercise-induced increase in interstitial [K\(^+\)] returned to pre-exercise levels by 15 min recovery, interstitial [Na\(^+\)] remained below pre-exercise levels by 25 min recovery (28).

Definitive studies on the precise mechanisms underlying fatigue-related alterations in muscle spindle and Golgi tendon organ afferent activity and investigations to confirm the contributory presence of a whole-body exchangeable sodium deficit, contracted interstitial fluid compartment, and hypersensitive neuromuscular junctions with sweat induced muscle cramping during exercise have not yet been performed.

To study this question, some physiological factors are considered. First, examining skeletal muscle immediately following exhaustive exercise may cause mitochondria swelling and cristolysis (27, 28) rendering impracticable the study of subtle changes in mitochondrial morphology.

Based on in vitro studies indicating that energy deprivation can induce mitochondrial fusion and elongation (29, 30); our initial hypothesis was that the state of metabolic undersupply occurred during exercise and increased energy demand relative to supply, may provoke the elongation and enlargement of mitochondria.
Picard et al., 2013 proved that mitochondrial dynamics in skeletal muscle fibers may serve different potential functional roles contributing to normal mitochondrial function and skeletal muscle physiology (31).

Physical tethers between mitochondrial membranes could promote the exchange of molecules (ions, lipids, proteins) between adjacent organelles (32, 33), which may help to coordinate the functioning of otherwise physically distinct mitochondria; protect healthy mitochondria from autophagy (31) without necessarily engaging energy-consuming processes of complete outer mitochondrial membranes (OMM) and inner mitochondrial membranes (IMM) fusion; and serve as perfusion events by tethering organelles’ outer membranes to facilitate their rapprochement and possible fusion upon the appropriate microenvironment signals. These speculative functions deserve further experimental clarification.

As defining the impact of fluctuations in energy metabolism on mitochondrial dynamics, both during exercise and sedentary behavior, has important implications for deciphering the mechanisms responsible for the health effects of physical in an activity (31, 34).

We can offer an advice for the athlete attempting to reverse a pattern of exertion heat cramping, it is often not necessary to increase fluid intake. Sometimes it’s essential to decrease fluid intake during and after activity for those who are overdrinking (especially those who are consuming too much low or no sodium fluid). The key is to increase sodium intake to more closely match individual sweat sodium losses, so that the appropriate amount of ingested fluid is better retained and distributed to all fluid compartments. The result is more complete rehydration (35).

**In conclusion**

From the present study it could be concluded that muscular exercise induced significant hyponatremia and Hyperkalemia in association with prominent histological alterations in skeletal muscles immediately after exercise. All these changes were ameliorated partially after rest. It is recommended for athletes to drink plain water better than drinking nothing, but drinking a properly for maintained carbohydrate electrolyte sports drink will allow better exercise performance.

**References**

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