Original Article / *Orijinal Makale*

DOI: 10.4274/tftr.05924

Turk J Phys Med Rehab 2013;59:229-35
Türk Fiz Tıp Rehab Derg 2013;59:229-35

After an Exhaustive Exercise the Most Prominent Muscle Damage Occurs a Day Later

Tüketici Bir Egzersiz Sonrasında En Belirgin Kas Hasarı Bir Gün Sonra Ortaya Çıkar

Elçin TAYLAN DEVEDEN, Lamia PINAR, Eser GÖKTAŞ*, Arzu L. ARAL**, Çiğdem ÖZER, Deniz ERDOĞAN

Gazi University Faculty of Medicine, Department of Physiology, Ankara, Turkey

**Gazi University Faculty of Medicine, Department of Histology and Embriology, Ankara, Turkey*

***Gazi University Faculty of Medicine, Department of Immunology, Ankara, Turkey*

Summary

Objective: In this research, long-term exercise-trained and untrained rats underwent exhausting exercise. We compared muscle damage, interleukin-6 (IL-6) - a product of the muscle cell myoblast and satellite cells in response to muscle injury -,free oxygen radicals that have been reported to be responsible for this damage and, antioxidant levels in both groups.

Materials and Methods: After exhausting exercise, trained and untrained rats were sacrificed by taking blood intracardiacally, just after exhaustion, one day and three days following exhaustion. Muscle damage was examined by light microscopy; the immune reactivity of the muscles was examined by IL-6 immunohistochemical evaluation and malondialdehite. Glutathione peroxidase levels of the muscles were assessed spectrophotometrically.

Results: After exhaustion, the muscle damage was found to be higher in the untrained group than in the trained group. Maximum muscle damage, IL-6 immunoreactivity and oxidant levels emerged one day after exhaustion in both trained and untrained groups. The drop of oxidants, increase in antioxidants and the visualized regeneration process in histological samples appeared more significantly on day three after the exhaustion in trained animals compared to untrained animals. Three days after exhaustion, IL-6 and oxidant levels were found to decrease, and especially in the trained group, it approached approximately to the level of controls. In the acutely running untrained group, although IL-6 involvement decreased, plasma oxidant levels were still found to be significantly higher and antioxidant levels were lower compared to that in controls.

Conclusion: This experiment revealed that the muscles of the exercise-trained rats were more resistant to this type of destructive muscle contraction than untrained rats. The IL-6 levels did not prominently increase just after the exhaustion but one day after exhaustion which made us think that the pro-inflammatory factors might have been suppressed by another agent - most probably by cortisol - in the beginning of the muscle damage and increased after the diminishing effects of these agents. *Turk J Phys Med Rehab 2013;59:229-35.*

Key Words: Exhausting exercise, myokines, muscle damage, muscle oxidants, muscle antioxidants

Özet

Amaç: Bu araştırmada, uzun süreli egzersiz eğitimi yapılmış sıçanlarla egzersiz eğitimi yapılmamış sıçanlar tüketici egzersize zorlanmışlardır. Egzersiz sonrasında her iki grupta; kaslarda meydana gelen hasar, hasara karşı gelişen sitokin yanıtı olarak interlökin-6 (IL-6) artışı ve bu hasardan sorumlu olduğu ileri sürülen serbest oksijen radikalleri ile antioksidan düzeyleri karşılaştırılmıştır.

Gereç ve Yöntem: Sıçanlar tüketici egzersizden hemen sonra ve egzersizi takip eden birinci ve üçüncü günlerde intrakardiyak kan alınarak sakrifiye edilmiştir. Kas hasarı ışık mikroskobu ile, IL-6 reaktivitesi immünohistokimyasal metod ile, malondialdehit ve glutatyon peroksidaz düzeyleri ise spektrofotometrik olarak değerlendirilmiştir.

Bulgular: Tüketici egzersiz sonrasında kas hasarı, egzersiz eğitimi yapılmamış grupta daha yüksek bulunmuştur. Kas hasarı, IL-6 immünoreaktivitesi ve oksidan düzeyleri her iki grupta da birinci günde en yüksek düzeylere ulaşmıştır. Egzersiz eğitimi yapılmış sıçanlarda oksidan düzeylerindeki azalma, antioksidan düzeylerindeki artış ve histolojik örneklerde izlenen iyileşme sürecine ait bulgular, tüketici egzersizden sonraki üçüncü günde daha belirgin hale gelmiştir. Tükenmeden üç gün sonra IL-6 ve oksidan düzeylerinin egzersiz eğitimli sıçanlarda azaldığı ve kontroller düzeyine yaklaştığı saptanmıştır. Akut koşturulan egzersiz eğitimi almamış grupta ise, IL-6 tutulumu azalmakla birlikte, plazma oksidan düzeyleri kontrollere nazaran anlamlı olarak yüksek; antioksidan düzeyleri düşük kalmaya devam etmiştir.

Sonuç: Bu çalışma, egzersiz eğitimi yapılmış olan hayvanların kaslarının bu şekilde bir hasar verici kas kontraksiyonuna, egzersiz eğitimi yapılmayanlara göre daha dirençli olduğunu ortaya koymaktadır. IL-6 düzeylerinin tüketici egzersizden hemen sonra değil, birinci günün sonunda belirgin şekilde artıyor olması, inflamasyonu baskılayıcı bazı ajanların -büyük olasılıkla da kortizolün- tüketici egzersizden hemen sonra aktive olarak egzersizin kaslar üzerinde hasar oluşturucu etkisi ile birlikte görülen pro-enflamatuvar faktörlerin artışını geciktirdiğini akla getirmektedir. *Türk Fiz Tıp Rehab Derg 2013;59:229-35*.

Anahtar Kelimeler: Tüketici egzersiz, miyokinler, kas hasarı, kas oksidanları, kas antioksidanları

Address for Correspondence:/Yazışma Adresi: Lamia Pınar MD, Gazi University Faculty of Medicine, Department of Physiology, Ankara, Turkey
Phone: +90 312 202 47 17 E-mail: lpinar@gazi.edu.trR eceived/Geliş Tarihi: February/Şubat 2012 Accepted/Kabul Tarihi: August/Ağustos 2012

Introduction

An effective physical activity induces a series of changes in the immune system, similar to the acute subclinical inflammation response (1). It is known that the production of free oxygen radicals increased in the muscles and plasma during and following a highly severe contractile activity leads to muscle damage (2). Free radicals are also claimed to initiate the antioxidant mechanisms, regulating the regeneration and adaptation of the muscle to exercise by activating the transcription factors sensitive to redox. The acute phase response activates the production of oxygen radicals together with various cytokines like interleukins (IL) and tumor necrosis factor–α (TNFα). The most prominent cytokine produced during the contractions is IL-6 which is expressed within the muscle cells and released into the blood, thus, it is defined as "myokine" due to its various endocrine and paracrine effects (3). Myokines regulate many endocrine and metabolic functions, thus, bring forward the effects of exercise on the organism (4). IL-6 activates the neutrophils for phagocytosis and triggers the oxidants, but concomitantly stimulates the antioxidant process during the formation of the acute phase proteins (5). Although its levels increase under inflammatory conditions, IL-6 has also anti-inflammatory properties (3).

The test protocol used in the majority of the studies investigating the 'immune response to contraction' was long-lasting exhausting exercise (6), as no increase in the interleukin levels was detected within the muscles or blood plasma with mild or moderate training (7).

In this study, we aimed to highlight the damage that would occur in the limb muscles in rats due to exhausting exercise, and the relationship between myokines and free radicals that are responsible for the induction of this injury and the difference, if any, between the aforementioned factors when the rats were exercise-trained (trained) and were made to run acutely (untrained). Both groups were further divided into groups to compare the factors known to be effective in the muscle damage and their relationship with time.

Materials and Methods

The study was approved by the Gazi University Ethics Committee for Animals, with the report no. 142-18805, and confirmed by the Guide of Institutional Animal Care of the Gazi University Experimental Research Centre.

Animals and Study Design

Three-month-old male Wistar Albino rats (n: 48) were housed in standard conditions (21±2°C, 12h light/12h dark cycle) with free access to tap water and food pellets. The rats were randomly assigned to 7 groups as: sedentary controls (C) that had never run in the experiment; untrained groups that were acutely forced to exhausting exercise and sacrificed; immediately after exhaustion (UT-i), 1 day after exhaustion (UT-1), 3 days after exhaustion (UT-3); trained groups, that had long-term exercise training, forced to exhausting exercise and sacrificed; immediately after exhaustion (T-i), 1 day after exhaustion (T-1) and 3 days after exhaustion (T-3).

At least six animals' data were gathered in each group. However, considering the risky effects of long-term exercise, the subgroups of the trained group consisted of eight animals.

Exercise Training and Adaptations on the Rats' Treadmill

Exercise-trained rats (T) performed 30 minutes treadmill running, five days a week for 12 weeks (8). Each session included warming up and cooling down periods (running 5m/min, 0º incline). Between these periods they were forced to run at 15m/min speed, 15º incline for 20 min (6,8). Untrained rats (UT) were adapted to the treadmill by running 5 m/min speed, 0º incline for 10 min over five days. A day after these exercise periods ended, both groups were forced to do exhausting exercise.

Exhausting exercise: Animals were forced to run at a rate of 20 m/min on a 0º incline, until they refused any further running, laid on their backs and failed to respond to an electrical shock of about 1.5 mA. The exhaustion time of each was recorded (9).

Experimental groups that consist of immediately after the exhaustion, one day after the exhaustion, and three days after the exhaustion groups and controls were sacrificed under ketamine hydrochloride (45 mg/kg) and xylazine hydrochloride (5 mg/kg) anesthesia, by taking blood intracardiacally. Plantar muscles were removed from both legs and kept at -80º C until analysis (10).

Histological Procedure

Muscles were sectioned at 4µm thickness repeatedly. Sections were stained initially with hematoxyline and eosin to investigate the morphology of myofibers under light microscopy.

Additional serial sections were stained for immunohistochemistry using peroxidase-anti-peroxidase (PAP) method with a goat polyclonal antibody against mouse IL-6 (M-19, sc: 1265, Lot: G 3008, Santa Cruz) and slides were examined with a photo-light microscope (DCM4500 Image Analyze System and QWin V3 Programme, Leica, Germany) in Gazi University Faculty of Medicine Histology and Embriology Laboratories. Scoring of immunoreactivity in stained tissues calculated semi quantitatively by the percentage of retention density in each group muscles as H score (HS) and classified as 0 (0, no retention), 1 (+, weak immunoreactivity), 2 (++, medium immunoreactivity), 3 (+++, strong immunoreactivity). Immunoreactivity for each area are summated and formulated as $\sum$ Pi.(I+1) (Pi: the percentage of retention; I: the density of retention).

Determination of malondialdehyde (MDA) and glutathione peroxidase (GSH) levels in muscles:

The muscle samples were obtained and frozen immediately in liquid nitrogen and kept in a -80ºC freezer until the analyses. Lipid peroxidation was quantified by measuring the formation of thiobarbituric acid reactive substances (TBARS). Lipid peroxide levels are expressed in terms of MDA equivalents using an extinction coefficient of 1.56×10^5 $mol^{-1}cm^{-1}$ (11).

The GSH levels were determined by a modified Elman method (12). The GSH levels were calculated using an extinction coefficient of 13.600 $mol^{-1}cm^{-1}$. Measurements of MDA and GSH were carried out at room temperature using a spectrophotometer (UV 1208, Shimadsu, Japan) in Gazi University Faculty of Medicine Physiology Laboratories.

Statistical Analysis

The mean exhaustion times of the untrained and trained groups were compared by using the Mann-Whitney U test and p value of less than 0.001 was considered statistically significant.

The values obtained in the immediately sacrificed group, one day after, three days after and the control groups were evaluated by using the Kruskal-Wallis test. Any differences established between the groups were evaluated by the use of the Bonferroni Correction of the Mann-Whitney U test and $p<0.0125$ was accepted as significant for corrected analyses. The dual comparison of the immediate, one day after, three days after and the controls of the chronic and acute groups were performed with the use of the Mann-Whitney U test and $p<0.05$ was accepted as significant.

Results

In this study, muscular structures and IL-6 immunoreactivity in the control group were found to be normal (Figure 1,2). The data indicating the muscle damage, IL-6 immunoreactivity and oxidants both in the UT and T groups were found to be the highest one day after exhaustion (Figure 3,4; Table 1). Muscle damage (Figure 3) and IL-6 immunoreactivity (Figure 4) were higher in UT groups than in the T groups (Table 1). Degenerative findings (Figure 3) and IL-6 immunoreactivity (Figure 4) in muscle fibers both in the T and UT groups attenuated three days after the exhaustion (Table 1).

Histopathological Investigation Showing Muscle Damage

The findings including the separations between the myofibrils, increased connective tissue between the muscle fibers, loss of striations and the circular formation of the nuclei that were transferred to the centrum beneath the sarcolemma were highest in the groups that were sacrificed one day after the exhausting exercise, both in the T group and UT group (Figure 3).

The signs pointing out the attenuation of muscle damage were found both in the T and UT groups as a decrease in

Table 1. Destructive effects of the exhausting exercise on rats' plantar muscles and the related IL-6 immunoreactivity of muscles involving trained (T) and untrained (UT) groups sacrificed immediately (-i), 1 day after (-1), 3 days after (-3) exhaustion.

Plantar muscle	Muscle fibers	Nuclei	Striation	Connective tissue	Figure No	IL-6 immuno-reactivity (HS*)	Figure No
Controls (C)	Normal	Beneath the sarcolemma, flat and ordered sequentially	Normal	Normal	Figure 1	Generally weak (HS: 16.3)	Figure 2
T-i	Parallel to each other Provide integrity	Beneath the sarcolemma, flat and ordered sequentially	Normal	Increased slightly	Figure 3	Weak moderate in sarcolemma, whole tissue and connective tissue (HS: 89.8)	Figure 4
T-1	Slight separations	Migrated towards the centre of the fibre. Circular/ spherical shape	Dense and light areas on myofibers	Increased	Figure 3	Strong in sarcolemma, whole tissue and connective tissue (HS: 306.8)	Figure 4
T-3	Slight separations	Migrated beneath the sarcolemma. Flat shape	Normal	Normal	Figure 3	Weak moderate in sarcolemma, whole tissue; weak in connective tissue (HS: 63.8)	Figure 4
UT-i	Slight separations	Migrated towards the centre of the fibre. Circular/spherical shape	Dense and light areas on myofibers	Increased	Figure 3	Moderate strong in sarcolemma, whole tissue and moderate in connective tissue (HS: 266.6)	Figure 4
UT-1	Increased separations	Migrated towards the centre of the fibre. Circular/spherical shape	Lack of striation. Dense and light areas on myofibers	Increased	Figure 3	Very strong in sarcolemma, whole tissue and connective tissue (HS: 395.6)	Figure 4
UT-3	Slight separations	Migrated towards the centre of the fibre. Circular/spherical shape	In some parts, lack of striation	Decreased compared to UT-1	Figure 3	Moderate in sarcolemma, whole tissue and connective tissue (HS: 134.6)	Figure 4

*HS: Mean H-score

oedema between the muscle fibres and a shift of the majority of the cell nuclei to the periphery in 'three days after exhaustion groups' (Figure 3).

Evaluation of IL-6 Immunoreactivity

The intensity of IL-6 immunoreactivity in muscles calculated by the percentage of retention is shown in Table 1 as mean H-scores (HS).

Immunohistochemical images showed a rise in IL-6 levels in the muscles of both the UT and T groups soon after immediately exhaustion (Figure 4, Table 1).

IL-6 immunoreactivity was highest on the next day after exhaustion, both in the UT and T groups, and decreased to approach the levels in control animals after three days (Figure 4, Table 1).

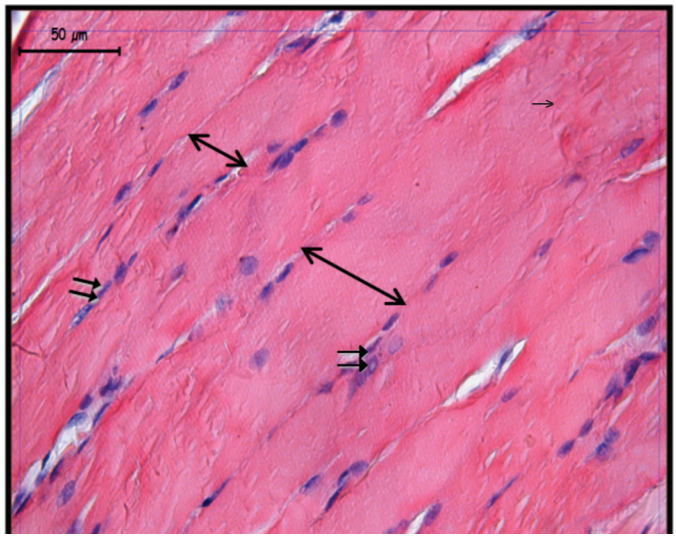

Figure 1. Muscular structures in the control group.→MF: Muscle fibers, ←N: Nucleus (Hematoxyline- and eosin X400).

Figure 2. IL-6 immunoreactivity in the control group (Immunoperoxidase-haematoxylin X400).

Assessment of Oxidants and Antioxidants

Muscle MDA and GSH levels are shown in Figure 5 and Figure 6, respectively.

- MDA levels were higher and GSH levels were significantly lower in UT groups than in T groups ($p<0.05$).

- Muscle GSH levels approached the control levels after the third day in the T group, however, the difference between the control group and the UT group was maintained within the same period of time.

- In untrained group, the difference in MDA levels between UT-i and UT-1 groups and between UT-1 and UT-3 groups were statistically significant ($p<0.001$) and this difference remained significant after Bonferroni correction.

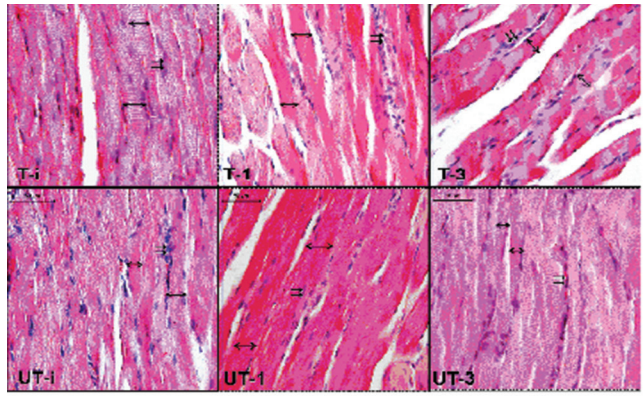

Figure 3. Muscular structures after exhausting exercise. T-i: Trained-immediate after exhaustion group. T-1: Trained-1 day after exhaustion group, T-3: Trained-3. day after exhaustion group, UT-i: Untrained-immediate after exhaustion group, UT-1: Untrained-1 day after exhaustion group, UT-3: Untrained-3 day after exhaustion group. ↔:Muscle fibers, ⇉: Nucleus (Hematoxyline-eosinX400).

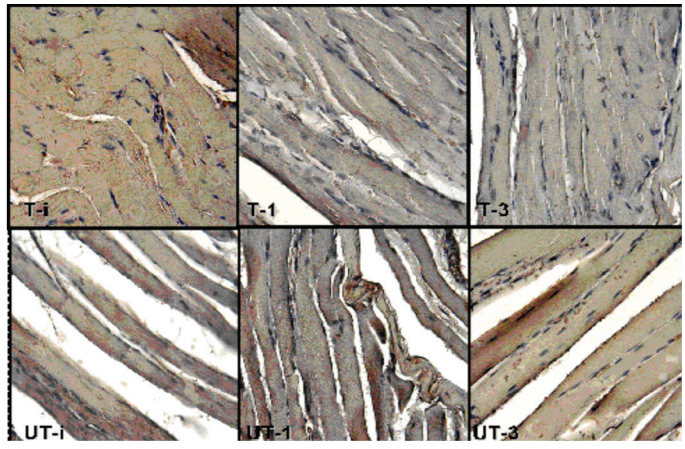

Figure 4. Immunohistochemical staining with the use of IL-6 antibody. T-i: Trained-immediate after exhaustion group, T-1: Trained-1 day after exhaustion group, T-3: Trained-3 day after exhaustion group, UT-i: Untrained- immediate after exhaustion group, UT-1: Untrained-1 day after exhaustion group, UT-3: Untrained-3 day after exhaustion group, (Immunoperoxidase-haematoxylinX400).

- In trained group MDA levels between T-i and T-1 groups and between T-1 and T-3 groups were found significantly different ($p<0.001$) and this difference remained significant after Bonferroni correction.

- MDA levels both in trained and untrained groups were found to be significantly higher than in control group ($p<0.01$).

- GSH levels were found to be significantly lower both in the trained and untrained groups compared to that in controls ($p<0.05$). In untrained group, the difference between the GSH levels measured immediately and after one hour measured were significant ($p<0.01$). In trained group, GSH levels measured immediately were statistically different than the levels measured after one hour and three hours ($p<0.01$). GSH levels (control group versus untrained group) measured after one hour and three hours and GSH levels (control group versus trained group) measured immediately and after one hour were significantly different ($p<0.01$).

Discussion

In this study, we tried to highlight the damage in rats' plantar muscle induced by exhausting exercise. We preferred studying the plantaris muscle as it is a fast muscle in the rats' limb (13) and is reported to have more prominent damage

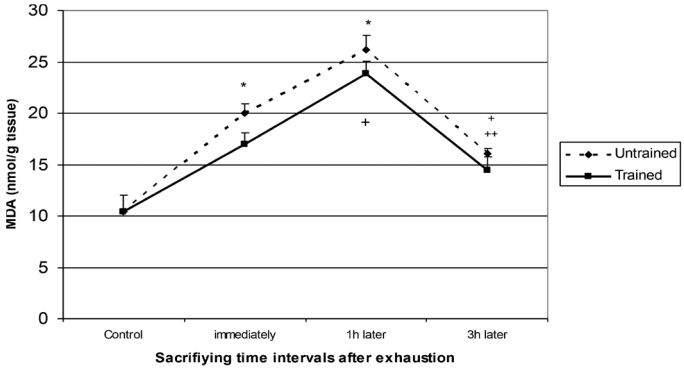

Figure 5. MDA values in plantar muscle tissue. Values are given as arithmetic mean ±SD. (*untrained vs. trained, immediately: $p<0.01$; 1h later: $p<0.05$; +untrained-immediately vs. 1h/3h later: $p<0.01$; ++trained-1h vs. 3h: $p<0.01$).

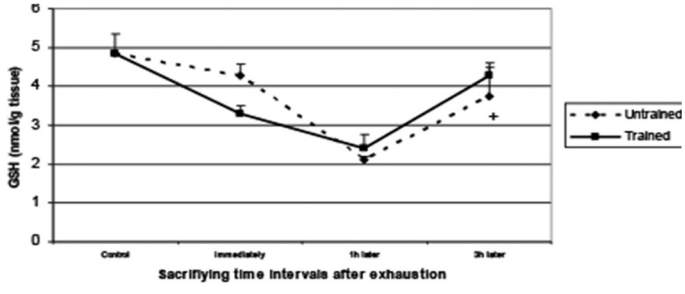

Figure 6. GSH values in plantar muscle tissue. Values are given as arithmetic mean ±SD. (*untrained vs. trained, immediately: $p<0.01$; + untrained-immediately vs. 1hour, trained-immediately vs. 1h/3h: $p<0.01$).

caused by forced exercise (14). IL-6 immunoreactivity, formed oxygen radicals and antioxidant levels in the muscle of the immediately after exhaustion, one day after exhaustion and three days after exhaustion sacrificed animals were investigated, along with the differences between the degree of damage formed in the muscles of T and UT groups following such forced exercise.

Severe and long-lasting exercise are known to give rise to a partial muscle damage (15). It has been shown that muscle soreness that emerge following an unaccustomed strong exercise progresses to reach a peak in 24-48 h (14). We were unable to reach to definite information concerning the reason why the damage and pain did not begin immediately after severe exhausting exercise but set off subsequently. In our study, the light microscopic and immunohistochemical analyses put forward that the most prominent muscle damage in untrained, acutely run, and long-term exercise-trained rats was observed to occur one day following the exhausting exercise. The oxidative parameters also indicated that the catabolic process in muscles was found to be higher one day after the exhausting exercises.

Histopathological investigation showed muscle damage such as separations between the myofibrils, increased connective tissue between the muscle fibers, loss of striations and the circular formation of the nuclei that were transferred to the centrum beneath the sarcolemma (16) to be highest in the next day following the exhausting exercise, both in the T group and more prominently in the UT group (Figure 3). The muscle damage in the subgroups of both groups immediately after exhaustion was relatively more severe when compared with the group that was investigated three days after exhaustion, however, the findings were found to be less severe when compared with the group that was assessed one day after the exhausting exercise (Table 1). Provided to be more in the trained group, the decrease in oedema in the muscles and the shift of the majority of the cell nuclei to the periphery in both the three days after exhaustion groups' assessments were taken as the indicators of healing (Figure 3). Similar to our findings, some researchers have also found high grade structural damage that consisted of dilatations and elongations in the Z bands after 24 h following an exhaustion exercise; they have also showed attenuation of these findings after 48 h and demonstrated the commencement of repair after 72 h (17).

The immune system generates a powerful and adaptive response a couple of hours after severe exercise with the effect of the stress hormones, muscle damage and inflammation leading to the secretion of cytokines (18). The factors that stimulate the liberation of cytokines are endotoxin leakage from the intestinal system throughout the exercise, increase in catecholamines and cortisol levels, increase in body temperature, and a deficit in glycogen together with other metabolic needs, and muscle damage (19).

Severe exercise increases the levels of pro- and anti-inflammatory cytokines (6). Recent studies have focused

primarily on IL-6 as the possible key molecule in muscle inflammation and repair (4).

We also thought that IL-6 could have a role during the inflammatory process in the enhancement of muscle damage or during the healing process. Immunohistochemical images showed a rise in IL-6 levels in the muscles of both the UT and T groups soon after exhaustion. These findings were concordant with the findings by Pedersen et al. (20), who put forward that the commencement of the inflammatory process and the rise in the IL-6 levels began within 30 min after the onset of exercise. However, in our study, parallel with the damage ensued, we found out that IL-6 immunoreactivity was highest on the next day after exhaustion, both in the UT and T groups, and decreased to approach the control animals' levels after three days (Figure 4). By investigating the literature, we found some studies which have stated that an IL-6 increase, arising from the muscles, is not linear, but is rather an exponential increase (21), reaching its maximum levels towards the end or soon after the end of the exercise, followed by a decrease towards the levels before the onset of exercise (22). Some researchers defended the idea that the half-life of IL-6 is three minutes, however, it continues to be actively released from the muscles for a couple of days after severe exercise (23). This literature search was unable to provide data indicating why the exercise induced muscle damage and IL-6 detention were highest one day after the exercise. As a result, we thought that there could be another factor, possibly the cortisol, which was stepping in and retarding the synthesis of IL-6 during acute exercise trauma. The literature states that the hypothalamo-pitutary-adrenal arch forms a response to the stress during acute and severe exercise. Adrenocorticotropic hormone and cortisol are the primary stress hormones that are released due to this kind activity. Cortisol provides the necessary energy material to the muscle and affects the immune system (24). There are studies indicating the inhibitor effects of cortisol on the peripheral production of IL-6 (25). This data enhances our assertion that the increased cortisol synthesis with the traumatic effect of exhausting exercise inhibited the IL-6 synthesis in the acute phase, and possibly as soon as the cortisol levels decreased, the muscle IL-6 levels increased. A study put forward that an increase in IL-6 levels leads to an increase in anti-inflammatory interleukins like IL-1ra and IL-10. Thus, IL-6 limits the potential harmful effects of inflammation and attenuates an inflammatory process (26).

One of the important results obtained from our study is that IL-6 detention was higher in the muscles of UT group than in the muscles of the T group. This finding is concordant with the knowledge found in the literature that describes the role of cytokines on the adaptation of muscles to exercise training (20). It has previously shown that IL-6 levels before and during exercise decrease with training (7). The mean exhaustion time of the rats was 149±35 min for the T group, while this was 84±26 min for the UT group rats, and the difference between them was statistically significant ($p<0.001$) which shows that the exercise training gave the rats physical endurance and an adaptation to exercise.

Increased levels of free radicals indicated that the metabolic breakdown and injury in the plantar muscle was significantly higher in rats that were sacrificed one day after exhaustion in comparison with the other groups in untrained rats, muscle MDA levels were significantly higher (Figure 5); GSH levels were lower (Figure 6) than in trained rats. GSH values were also found to be lowest in both groups when assessed one day after exhaustion. There is no doubt that reactive oxygen species increase during exercise (27). Long-term exercise, however, augments the protection against oxidative stress and provides a high resistance for the body. The accommodation process to exercise contains incidents like the activation of antioxidant system, redox-sensitive transcription factors, gene expression and protein synthesis. This leads to an increase in DNA repair mechanisms and proteosome complexes during the accommodation period. Molecular accommodation facilitates resistance to oxidative stress and development in physiological functions (27). The trained subjects demonstrated less oxidative injury when compared with the untrained subjects following exhausting exercise. This situation is well explained by the significant up-regulation of the endogenous antioxidants (28). Eventually, the accommodation response can be a cumulative result of repeated exercises, thus, because the tissues are not ready for the acute exhausting exercise they get damaged (29).

In our study, MDA levels were found to be significantly higher in UT groups when compared with T groups (Figure 5). Accordingly, all MDA levels in the rat subgroups that run acutely were found to be higher and the GSH levels were significantly lower when compared with their equivalent groups of trained rats. The antioxidant capacity in T-1 and T-3 groups was significantly higher compared to the UT-1 and UT-3 groups (Figure 6). This situation may be effective in the attenuation of the oxidative stress that would have been induced by the exhausting exercise in the trained group. In a similar study, superoxide dismutase and glutathione S-transferase, which are accepted to be the indicators of the antioxidant markers in plasma, have prevented the rise of lipid peroxidation, the indicator of oxidative stress (30).

In conclusion, our study showed that the degeneration process in the muscles intensified one day after exhausting exercise in both trained (long-term exercised) and untrained (acutely run) groups. However, the regeneration process began to develop three days after exhaustion in trained rats, but the regeneration signs were not significant in untrained rats three days after exhaustion. We also found that IL-6 immunoreactivity in the rat plantar muscles exposed to vigorous contractions and damage mostly exacerbated one day after exhaustion, and lowered in about three days. This experiment related with muscle damage after exhausting exercise also revealed that the muscles of the exercise-trained rats were more resistive to this type of destructive muscle contraction than untrained rats. The IL-6 levels that did not prominently increased just after the exhaustion but one day after exhaustion made us think that the pro-inflammatory factors might be suppressed by another agent -most probably by cortisol- in the beginning of the muscle damage and increased after the diminishing effects of these agents. Our next aim is to research the causes of the delayed increase in IL-6, oxidants and delayed muscle soreness occurring after the exhausting exercise.

References

1. Rosa Neto JC, Lira FS, Oyama LM, Zanchi NE, Yamashita AS, Batista ML Jr, et al. Exhaustive exercise causes an anti-inflammatory effect in skeletal muscle and a pro-inflammatory effect in adipose tissue in rats. Eur J Appl Physiol 2009;106:697-704.
2. Roebuck KA. Oxidant stress regulation of IL-8 and ICAM-1 gene expression: differential activation and binding of the transcription factors AP-1 and NF-kappaB. Int J Mol Med 1999;4:23-30.
3. Nielsen S, Pedersen BK. Skeletal muscle as an immunogenic organ. Curr Opin Pharmacol 2008;8:346-51.
4. Pedersen BK, Febbraio M. Muscle-derived interleukin-6: A possible link between skeletal muscle, adipose tissue, liver, and brain. Brain Behav Immun 2005;19:371-6.
5. Niess AM, Sommer M, Schneider M, Angres C, Tschositsch K, Golly IC, et al. Physical exercise-induced expression of inducible nitric oxide synthase and heme oxygenase-1 in human leukocytes: effects of RRR-alpha-tocopherol supplementation. Antioxid Redox Signal 2000;2:113-26.
6. Ostrowski K, Rohde T, Asp S, Schjerling P, Pedersen BK. Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. J Physiol 1999;515:287-91.
7. Fischer CP. Interleukin-6 in acute exercise and training: what is the biological relevance? Exerc Immunol Rev 2006;12:6-33.
8. Spier SA, Delp MD, Meininger CJ, Donato AJ, Ramsey MW, Muller-Delp JM. Effects of aging and exercise training on endothelium-dependent vasodilatation and structure of rat skeletal muscle arterioles. J Physiol 2004;556:947-58.
9. Koçtürk S, Kayatekin BM, Resmi H, Açıkgöz O, Kaynak C, Özer E. The apoptotic response to strenuous exercise of the gastrocnemius and soleus muscle fibers in rats. Eur J Appl Physiol 2008;102:515-24.
10. Greene EC. Anatomy of the rat. New York: Hafner Publishing Company; 1963.
11. Casini A, Ferrali M, Pompelam A, Maellaro A, Comporti M. Lipid peroxidation and cellular damage in extrahepatic tissues of bromobenzene intoxicated mice. Am J Pathol 1986;123:520-31.
12. Aykaç AG, Uysal M, Yalçın AS, Koçak-Toker N, Sivas A, Öz H. The effect of chronic ethanol ingestion on hepatic lipid peroxide, glutathione, glutathione peroxidase and glutathione transferase in rats. Toxicology 1985;36:71-6.
13. Dunn SE, Simard AR, Bassel-Duby R, Williams RS, Michel RN. Nerve activity-dependent modulation of calcineurin signaling in adult fast and slow skeletal muscle fibers. J Biol Chem 2001;276:45243-54.
14. Levy MN, Koeppen BM. Berne&Levy Principles of Physiology. 5th ed. Philadelphia: Mosby Elsevier; 2008.
15. Zuo L, Christofi FL, Wright VP, Bao S, Clanton TL. Lipoxygenase-dependent superoxide release in skeletal muscle. J Appl Physiol 2004;97:661-8.
16. Pedersen BK, Akerstrom TC, Nielsen AR, Fischer CP. Role of myokines in exercise and metabolism. J Appl Physiol 2007;103:1093-8.
17. Su Q-S, Zhang JG, Dong R, Hua R, Sun JZ. Comparison of changes in markers of muscle damage induced by eccentric exercise and ischemia/reperfusion. Scand J Med Sci Sports 2009;20:748-56.
18. Suzuki K, Yamada M, Kurakake S, Okamura N, Yamaya K, Liu Q, et al. Circulating cytokines and hormones with immunosuppressive but neutrophil-priming potentials rise after endurance exercise in humans. Eur J Appl Physiol 2000;81:281-7.
19. Nieman DC, Henson DA, Smith LL, Utter AC, Vinci DM, Davis JM, et al. Cytokine changes after a marathon race. J Appl Physiol 2001;91:109-14.
20. Pedersen BK, Febbraio MA. Muscle as an Endocrine Organ: Focus on Muscle-Derived Interleukin-6. Physiol Rev 2008;88:1379-406.
21. Steensberg A, van Hall G, Osada T, Sacchetti M, Saltin B, Klarlund Pedersen B. Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6. J Physiol 2000;529:237-42.
22. Ostrowski K, Rohde T, Zacho M, Asp S, Pedersen BK. Evidence that interleukin-6 is produced in human skeletal muscle during prolonged running. J Physiol 1998;508:949-53.
23. Castell JV, Geiger T, Gross V, Andus T, Walter E, Hirano T, et al. Plasma clearance, organ distribution and target cells of interleukin-6/hepatocyte-stimulating factor in the rat. Eur J Biochem 1988;177:357-61.
24. Borer KT. Exercise Endocrinology. 2nd ed. Champaign (Illinois): Human Kinetics; 2003.
25. Papanicolaou DA, Tsigos C, Oldfield EH, Chrousos GP. Acute glucocorticoid deficiency is associated with plasma elevations of interleukin-6: does the latter participate in the symptomatology of the steroid withdrawal syndrome and adrenal insufficiency? J Clin Endocrinol Metab 1996;81:2303-6.
26. Steensberg A, Fischer CP, Keller C, Moller K, Pedersen BK. IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. Am J Physiol Endocrinol Metab 2003;285:433-7.
27. Alessio HM, Goldfarb AH. Lipid peroxidation and scavenger enzymes during exercise: adaptive response to training. J Appl Physiol 1988;64:1333-6.
28. Andrade FH, Reid MB, Westerblad H. Contractile response of skeletal muscle to low peroxide concentrations: myofibrillar calcium sensitivity as a likely target for redox-modulation. FASEB J 2001;15:309-11.
29. Calabrese EJ. Hormesis: a revolution in toxicology, risk assessment and medicine. EMBO Rep 2004;5:37-40.
30. Souza-Rabbo MP, Silva LF, Auzani JA, Picoral M, Khaper N, Belló-Klein A. Effects of a chronic exercise training protocol on oxidative stress and right ventricular hypertrophy in monocrotaline-treated rats. Clin Exp Pharmacol Physiol 2008;35:944-8.