Annals of Plastic and Reconstructive Surgery

Research Article
Published: 13 Jul, 2023

The Effect of Pulsed Electromagnetic Fields on Rat Muscle Ischemia-Perfusion Model

Yuvacı AU[1], Yavuz OM[1], Pelit A[2], Taştekin B[2] and Sapmaz T[3]*

[1]*Department of Plastic, Reconstructive and Aesthetic Surgery, Cukurova University, Turkey*

[2]*Department of Biophysics, Cukurova University, Turkey*

[3]*Department of Histology and Embryology, Cukurova University, Turkey*

Abstract

Introduction and Aim: Ischemia is defined as the reduction or cessation of blood flow to a tissue. Reperfusion is maintaining the blood flow to the tissue again. These changes in blood flow to the tissues cause damage. This damage depends on the cause, duration, and severity of ischemia, as well as reperfusion. It is so-called Ischemia-Reperfusion (I-R) injury. Although I-R injury has a complex pathophysiological process accompanied by many mechanisms, the elucidation of these mechanisms has led to a search for solutions in order to prevent the damage. Despite numerous surgical and pharmacological studies, a safe treatment that can reverse I-R damage has not been found yet. Pulsed Electromagnetic Field (PEMF) therapy is a treatment modality that has been used for many years and has proven its effectiveness on bone healing, wound healing, tissue regeneration, and anti-inflammatory properties. Our study aims to examine the effects of PEMF application on skeletal muscle damage after I-R injury in the lower extremity of the rat.

Material and Method: 40 male Wistar-Albino rats, aged between 4 to 6 months, with a starting weight of 250 g to 350 g, were divided into 4 different groups. Group 1 was the control group and no application was made (n=10). In groups 2, 3, and 4, the blood flow of the lower extremities of the rats was stopped in accordance with the hindlimb ischemia method by constricting the femoral artery-vein system with micro clips, and ischemia was created for 4 h. Reperfusion was achieved after 4 h. Group 2 did not receive any treatment (n=10). In Group 3, PEMF treatment was applied for 1 h per day for 30 days after I-R damage was created (n=10). In Group 4, PEMF treatment was applied 2 h per day for 30 days after I-R damage was created (n=10). After 30 days, the lower extremities of the rats were evaluated macroscopically by Ischemia Score. After the application of Indocyanine Green (ICG) from the tail vein, the lower extremity perfusion was visualized and mapped with the SPY device. After the evaluation, the rats were sacrificed and samples were taken from the gastrocnemius muscles. The samples were examined under a light microscope and electron microscope. In addition, in order to examine the muscle functions biophysically, the isometric contraction forces of the muscles were evaluated by taking them into an isolated organ bath.

Results: As a result of the macroscopic evaluation according to the ischemia score, all groups were evaluated as normal. Percentage mapping was performed in the imaging performed on the SPY device, and it was observed that the perfusion in Group 3 and Group 4 was better than Group 2 and similar to Group 1. In the light microscope, it was observed that the leukocyte density decreased in Group 3 and Group 4, and the decrease in Group 3 was more in the comparison between these groups. It was observed that lymphocyte density increased in all groups in which I-R was created. Neovascularization was evaluated by capillary density, and it was observed that it increased significantly in Group 3 and Group 4, where PEMF was applied. In the electron microscopy examination, reversal of muscle degenerations in Group 3 and Group 4 was observed, and the morphology in Group 4 was similar to Group 1. A statistically significant difference was observed when the contraction forces were compared in the evaluation of isometric muscle contraction ($p<0.01$). Group 1 and Group 4 were observed to have similar strengths. When the contraction times were compared, a significant difference was observed between the groups ($p<0.024$). There was no statistically significant difference between relaxation times.

Conclusion: There are many studies on the effects of PEMF application in the literature, and healing effects on tissues, especially on bone, and anti-inflammatory effects have been demonstrated. However, the limited number of studies on I-R injury and the fact that these studies did not evaluate the loss of function after I-R injury makes our study different. PEMF application can be considered

OPEN ACCESS

Correspondence:
Ahmet Umut Yuvacı, Department of Plastic, Reconstructive and Aesthetic Surgery, Cukurova University, 01330 Saricam, Adana, Turkey, Tel: +903223386060-3226; Fax: +903223386900

Received Date: *22 Jun 2023*
Accepted Date: *04 Jul 2023*
Published Date: *13 Jul 2023*

Citation:
Yuvacı AU, Yavuz OM, Pelit A, Taştekin B, Sapmaz T. The Effect of Pulsed Electromagnetic Fields on Rat Muscle Ischemia-Perfusion Model. Ann Plast Reconstr Surg. 2023; 7(1): 1105.

as a treatment option because it has effects on many pathways involved in the mechanism of I-R injury, has accelerating effects in the recovery process after the injury, and has no systemic side effects. In addition, the fact that it is effective in restoring the loss of strength in the skeletal muscle after I-R injury makes PEMF treatment different from all other treatment modalities.

Keywords: Ischemia reperfusion injury; Pulsed electromagnetic field; Skeletal muscle

Introduction

Insufficient or cessation of blood flow to an organ is called ischemia. Ischemia is the first part of the ischemia-reperfusion injury and is characterized by the shift of cellular metabolism to the anaerobic side [1]. The resumption of oxygenation with the restoration of blood flow is called reperfusion. Metabolites released by anaerobic metabolism are oxidized after the start of reperfusion, and they enter the circulation and cause distant organ damage. The clinical condition resulting from this process is called Ischemia-Reperfusion (I-R) injury. I-R injury is characterized by an increase in Free Oxygen Radicals (FOR) and inflammatory molecules (TNF-α, IL-1, IL-6), activation of the complement system, endothelium-dependent vasodilation dysfunction, leukocyte-endothelium-platelet adhesion, and increase in microvascular permeability. The continuation of leukocyte migration to the tissue remaining in ischemia after reperfusion causes I-R damage to continue and progress further. The deleterious effects that occur with the onset of reperfusion to the ischemic remaining viable tissue are broader and more serious than ischemic injury.

The severity of ischemia-related damage is proportional to the duration and amount of ischemia and differs according to the cell type, susceptibility to injury, differentiation, blood requirement, and metabolism. The critical ischemia time refers to the maximum time the tissue can tolerate complete ischemia and remain viable after circulation is restored. The mean time to critical ischemia is the ischemic period that causes 50% of flaps to die [1]. Irreversible damage occurs in the muscle tissue in the event of ischemia exceeding four hours. It is thought that when the inflammatory response in skeletal muscle ischemia-reperfusion injury is suppressed, organ damage will also decrease.

Pulsed Magnetic Field (PEMF) therapy is a non-invasive treatment method based on magnetic field interaction, which is a natural and sensitive form of treatment. The fact that it has a wide application area, is an easy application, is a natural method, and has no side effects makes this treatment method important. Although there are experimental and clinical findings showing that PEMF has positive effects on bone healing, wound healing, healing of vascular structures, and healing of burn wounds, studies on tissues such as skin, muscle, and tendon are not sufficient.

Our study aims to analyze the pathological, biomechanical, and histological effects of PEMF on I-R damage in the gastrocnemius muscle when applied at different durations.

Material and Method

Animals

This study was carried out with the support of Çukurova University Scientific Research Projects fund with the approval of Çukurova University Presidency of Experimental Animals Ethics Committee dated 04-02-2019 and project number TTU-2020-12886. In this study, healthy male Wistar albino rats aged between 4 to 6 months and starting weights of 250 g to 350 g were used. Selected animals have not been used for other experiments before. Rats were provided by Çukurova University Health Sciences Experimental Application and Research Center. Animals were kept in steel cages, with a maximum of 5 animals in each cage, in accordance with ethical rules throughout the research. During this period, the animals were able to drink as much water and eat as they wanted. The room in which they are housed is arranged to be 12 h of light and 12 h of darkness. In addition, the room temperature of the laboratory was measured with a thermometer and humidity with a hygrometer, and it was constantly ventilated with an aspirator so that the temperature would not exceed 23 ± 1°C.

Acute hindlimb ischemia model

All surgical procedures were performed under sterile conditions and by the same surgeon under 10% ketamine HCL (50 mg/kg) and xylazine HCL (2.5 mg/kg) anesthesia. After making sure that sufficient depth of anesthesia was provided, the abdomen was shaved. While the rats were in the supine position, their extremities were fixed to the floor with tape. After shaving the rat abdomen, it was cleaned with sterile gauze impregnated with povidone-iodine. The femoral artery and vein were explored through an incision from the inguinal region. Ischemia was created by stopping femoral artery blood flow and femoral vein blood flow with micro clips (VASCU-STATT Single-use bulldog clamps, Minnesota, USA, gram pressure 50 to 60 max) (Figure 1). After it was observed that the blood flow stopped, the inguinal region was sutured with 4/0 Ethilon (Ethicon, Shanghai, China) and closed. After four hours of ischemia, the micro clips were opened and femoral artery and femoral vein blood flow was observed. The wound was sutured with 4/0 Ethilon. After 1 month, the rats were sacrificed by cervical dislocation after anesthesia, and samples were taken from the gastrocnemius muscle (Figure 2).

Pulsed electromagnetic field treatment

PEMF system was constructed using two Helmholtz coils with a diameter of 2r=60 cm and a circular structure with a spacing of r=30 cm. When connected to a programmable signal generator (ILFA Elektronik, Adana, Turkey), magnetic field output of desired frequency and intensity can be provided through these coils. The coils were placed in a (90 × 90 × 50) cm Faraday cage (Figure 3). A (30 × 30 × 25) cm plexiglass box, in which the rats were placed, was placed in the middle of the uniform magnetic field created perpendicular to

Figure 1: Clamping the femoral artery and vein with microclips.

Figure 2: Image of dissected gastrocnemius muscle.

Figure 3: Helmholtz Coils placed in the Faraday cage.

Figure 4: Rats placed in a plexiglass box between the coils.

the earth between the coils (Figure 4). The peak value of the magnetic field was measured with a Gaussmeter (F.W. Bell Model 6010, Sypris Company, Orlando, FL) used to measure the magnetic field with the help of a Hall effect probe in the Department of Biophysics. In the system for which preliminary studies were carried out, it was observed that the time-varying magnetic field consists of a semi-triangular waveform with a 0.5 ms rise time and a 9.5 ms fall time. The waveform of the induced electric field was measured using a probe coil probe (50 mm internal diameter, 50 turns, 30-gauge copper wire) placed on the mid-central axis of the Helmholtz coils. The probe tips were connected to an oscilloscope (Hitachi, Tokyo, Japan) and the induced voltage was directly read. The corresponding induced electric field was observed to be in the form of a unipolar rectangular wave with peak electric fields of 0.6 V/m (0.59 V/m to 0.61 V/m) in the holder located between the coils. The maximum induced electric field between the coils was calculated using Faraday's law. The current in the circuit (I=5.2 A) was also monitored on an oscilloscope through a resistor (0.1 Ω) connected in series between the output of the power

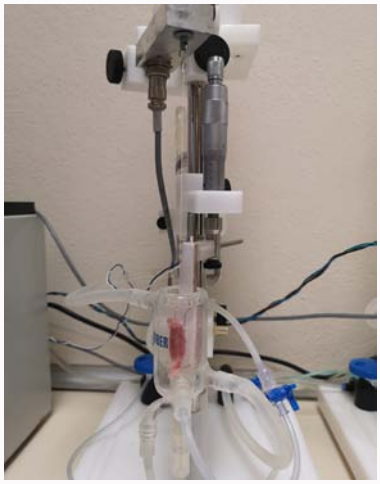

Figure 5: Gastrocnemius muscle suspended in the isolated organ bath.

Figure 6: Isolated organ bath and recording system.

amplifier and the coil. Helmholtz coils connected to a programmable power supply allow the production of the magnetic field at the desired intensity by adjusting the Pulse Level on the power supply. Controls were performed as described above.

Biophysics evaluation

A pre-prepared Krebs solution was used in the organ bath for biomechanical recordings. Krebs solution content; 118 mM NaCl, 4.69 mM KCl, 0.6 mM $MgSO_4$, 1.17 mM KH_2PO_4, 11.1 mM Glucose, 25.0 mM $NaHCO_3$ and 2.5 mM $CaCl_2$, and the pH was adjusted to ~7.4. The organ bath was continuously gassed with 95% O_2 + 5% Carbon Dioxide (CO_2).

After the rats were anesthetized with a combination of Ketamine HCl (80 mg/kg, Ketalar, Pfizer) and Xylazine (20 mg/kg, Rompun 2%, Bayer), they were subjected to the sacrificial process by pulling their tails and spinal cord injury. Afterward, the gastrocnemius muscles of the right leg were dissected. The muscles were tied with threads at their distal and proximal end, properly placed between platinum electrodes, and suspended in an isolated organ bath containing Krebs solution. The bath temperature was kept constant at 28°C (Figure 5).

After the optimum lengths of the gastrocnemius muscles of the control and magnetic field groups were found, they were directly stimulated supramaximally for 20 min with 0.5 ms duration (0.05 Hz) square pulses (15 V to 20 V) during the preparation period. Muscle responses with more than 20% of the supramaximal stimulus were recorded with an isometric force converter from the MP30 data

acquisition system to the computer. Pulse, frequency parameters, and derivative values were determined with Biopac Student Lab Pro v 3.6.7 program. Gastrocnemius muscles in each group were first directly stimulated with single supramaximal square pulses, and isometric jolt contractions were recorded. The concussion force (PT), Contraction Time (CT), and half-Relaxation Time (½RT) parameters were determined from the concussion curves recorded in the computer. From the derivative curve, the points (times) at which the contraction and relaxation rates are maximum can also be determined.

After ~30 min of thermoregulation and equilibration time, a single jolt was stimulated supramaximally with 1 Hz square pulses of 0.5 ms (15 V to 20 V) duration to record isometric contraction values. Contractile responses were recorded with square pulses with a frequency of 10 Hz, 20 Hz, 50 Hz, and 100 Hz (15 V to 20 V) of 0.5 ms duration, waiting ~30 min between applications. A force transducer (FDT 10-A 500 g, Commat, Ankara, Turkey) was used to record the force values obtained against muscle stimulation. Contractile responses were obtained in the form of muscle isometric contraction force (PT, g-force), Contraction Time (CT, ms), Relaxation Time (RT, ms). After the contraction responses were recorded, the moist weights of the muscles were recorded. Biopac MP30 device, MAY organ bath (MAY WBC3044), MAY ISO-150 stimulator, and MAY water heater circulator were used to record contraction forces and parameters. Data were analyzed with Biopac Student Lab Pro v 3.6.7 program (Figure 6).

Histopathologic evaluation

Tissues were fixed in a 10% formalin solution for 24 h. Tissues undergoing routine follow-up were embedded in paraffin. Sections of 4 µm thickness taken from paraffin blocks were stained with Hematoxylin and Eosin (H&E). All sections in the prepared stained preparations were examined under the Nikon Eclipse E 600 light microscope in the Department of Medical Pathology, Çukurova University. Each preparation was examined at x200 magnification.

Histopathological examination was performed by a pathologist, without knowing which tissue sample belonged to which group and by randomly selecting tissue samples. The sections are respectively; four parameters were taken into consideration, namely PMNL, lymphocyte density, capillary vessel proliferation, and fibrosis. While making pathological evaluations, the evaluation was made with these criteria; Leukocyte density: Normal: 0, Medium: 1, Intensive: 2; Lymphocyte density: Normal: 0, Medium: 1, Intensive: 2; Capillary vessel proliferation: None: 0, Lightweight: 1, Medium: 2, Intense: 3; Fibrosis: None: 0, Yes: 1.

Electron microscopic examination

The muscle tissue samples for electron microscopic evaluation were kept in a 5% glutaraldehyde solution prepared with Millonig's phosphate buffer for 3 h. The tissues were then shaken twice in the buffer for 10 min and then fixed for the second time in a 1% osmium tetroxide solution prepared with Millonig's phosphate buffer. After two hours of osmium tetroxide fixation, the tissues were washed twice with a phosphate buffer for 10 min. The tissues were then dehydrated in 50%, 70%, 86%, 96%, and 100%, ethyl alcohol respectively (for 15 min). The tissues were then treated in Propylene oxide (twice for 15 min) and in propylene oxide + resin for 30 min (twice). After these procedures, the tissue pieces were taken into tubes containing freshly prepared embedding material (resin) and mixed in a rotator overnight. The following day, tissue samples received from the rotator were embedded in Beem capsules using freshly prepared embedding material and polymerized in a 60°C drying oven for 48 h. Then, the ultrathin sections (50 nm in thickness) were cut using a Leica Reichert Ultracut S ultramicrotome (Austria). The sections were stained with saturated uranyl acetate prepared in 70% ethyl alcohol and lead citrate solutions. The stained sections were examined with the JEOL-JEM 1400 Transmission Electron Microscope (Japan) and their micrographs were obtained.

Figure 7: Tail vein injection.

Infrared fluorescent angiography perfusion evaluation

SPY (Novadaq Technologies; Toronto, ON, Canada) is an active device used to monitor the circulation in blood vessels and lymphatics as well as perfusion of related tissues with near-infrared fluorescence imaging during the operation. The working principle of the SPY system is as follows; In the imaging console, the Near-Infrared (NIR) light from the illumination module is transmitted to the imaging head with a fiber optic cable. The imaging head is placed on the patient so that the NIR excitation light comes over and illuminates the relevant area. When the patient is injected with "indocyanine green" (Indocyanine Green-ICG), this molecule binds to the plasma proteins in the blood and goes to the relevant area of the blood circulation. The NIR excitation light emitted by the SPY instrument imaging head causes the ICG to fluoresce. The fluorescent response of the ICG is then displayed by the NIR camera in the imaging head, and the resulting image signal is processed in the computer and simultaneously stored in the computer memory, and displayed in real-time on the video monitors. The normal biological life of ICG is 2.5 min to 3 min.

Animals were anesthetized with 10% ketamine HCL (50 mg/kg) and Xylazine HCL (2.5 mg/kg). The animals were fixed to the floor with plaster by abducting their extremities so that imaging could be performed on the SPY device. 0.1 ml, 0.5 mg/kg ICG was administered intravenously from the tail vein (Figure 7). Perfusion rates in the lower extremity muscles were visualized and mapped with the SPY device.

Statistical evaluation

Categorical variables were expressed as numbers and percentages, while continuous variables were summarized as mean and standard deviation (median and minimum-maximum where necessary). The normality of the distribution for continuous variables was confirmed by the Shapiro Wilk test. One-way ANOVA or Kruskal Wallis test

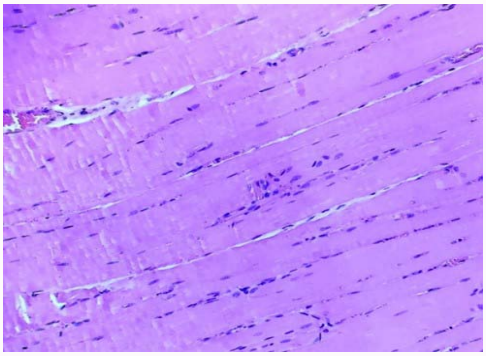

Figure 8: Slightly increased capillary proliferation in group 3 H&E x200.

was used in the comparison of more than two groups, depending on whether the statistical hypotheses were met or not. For normally distributed data, Bonferroni tests were used for multiple comparisons of groups regarding the homogeneity of variances. Bonferroni adjusted Mann Whitney U test was used for multiple comparisons of groups for data that did not show normal distribution. Chi-square test statistics were used to compare categorical measures between groups. All analyzes were performed using the IBM SPSS Statistics Version 20.0 statistical software package. The statistical significance level was accepted as 0.05 for all tests.

Results

Histopathological findings

Histopathologically, Group 1 shows findings within physiological limits. In group 2, leukocyte and lymphocyte density

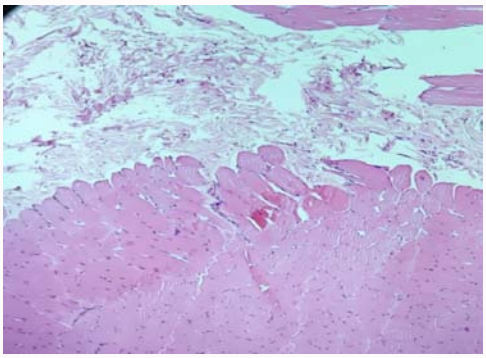

Figure 9: Fibrosis observed in group 2 H&E x100.

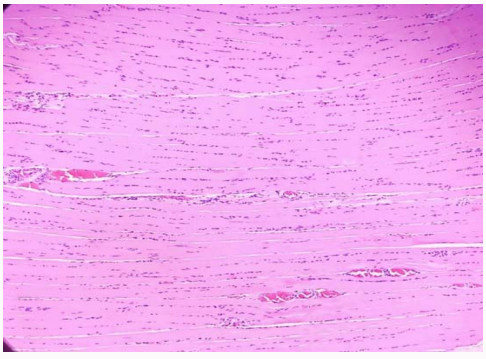

Figure 10: Capillary proliferation and chronic inflammation observed intensively in PMA2 H&E x100.

Table 1: Changes in leukocyte and lymphocyte density, capillary vessel proliferation, fibrosis parameters between groups.

Groups		Leukocyte Density	Lymphocyte Density	Capillary Proliferation
Group 1	1	-	++	+++
	2	+	+	++
	3	-	+	++
	4	+	+	++
	5	-	+	+
	6	+	+	++
	7	-	+	+
	8	-	+	+++
Group 2	1	+	+	++
	2	+	+	++
	3	+	+	+
	4	++	+	+
	5	-	+	+
	6	-	+	+
	7	-	+	+
	8	-	+	+
Group 3	1	-	-	-
	2	-	-	-
	3	-	+	-
	4	-	+	-
	5	-	+	-
	6	-	-	-
	7	-	-	-
	8	-	-	-
Group 4	1	-	+	-
	2	-	+	-
	3	++	++	+
	4	++	+	++
	5	++	++	+
	6	++	++	+
	7	++	++	+
	8	++	++	+

(inflammation) are remarkable compared to other groups. Capillary vessel proliferation observed in Group 4 was observed to be more prominent than other groups. Capillary vessel proliferation was slightly increased in Group 3 as well (Figure 8). The leukocyte density in Group 3 is higher than in Group 4. This shows that PEMF exposure time increases angiogenesis and anti-inflammation effects. The degree of fibrosis in Group 3 and Group 4 was less observed compared to Group 2 (Figure 9). PEMF directly and/or indirectly affects the function of cells that have a primary effect on wound healing, such as mononuclear cells and regenerative endothelial cells. The prominent capillary vessel proliferation observed in Group 4 not only exhibits the angiogenic effect of this process with endothelial proliferation, but the more pronounced inflammation in Group 2 indicates that it may also have an anti-inflammatory effect (Table 1 and Figure 10).

Electron microscope findings

Group 1 (control group): In the electron microscopic evaluation

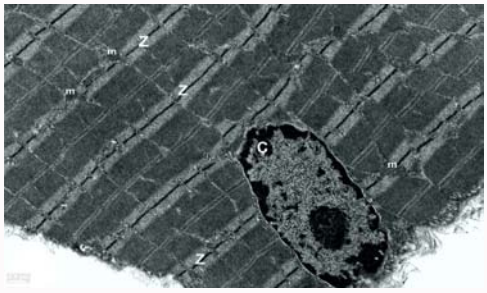

Figure 11: Electron microscopic view of striated muscle tissue of Group 1. Normal chromatin arrangement of the cell nucleus (C) is seen. Mitochondria (m) maintain their normal thin structure with their cristae and membranes. Sarcomeres, which are contractile units, are observed between the two Z lines. Bar: 0.5 µm.

Figure 12: Electron microscopic view of striated muscle tissue of Group 2. Heterochromatin increase is observed in the cell nucleus (C). Vacuoles of different sizes (v) are seen in the cytoplasm, including membranous whorl structures (w). Bar: 0.5 µm.

of the muscle tissues of the control group, which did not cause I-R damage and did not receive any treatment, it was observed that the skeletal muscle fibers were surrounded by sarcolemma externally, and the sarcolemma invaginated transversely into the cell at the A-I band junctions. The Z line, an electron dens transverse line, was seen in the middle of the I band. In the middle of the darker A band, the less intense H band and the M line located in the middle of this band were observed. It was observed that the sarcomere, which is the myofibril part between the two adjacent Z lines and called the contractile unit of the striated muscle, had a normal structure. It was observed that the muscle fibers had a well-developed sarcoplasmic reticulum, which formed a highly organized tubular network around the contractile elements (Figure 11).

Group 2 (experimental control group): In the electron microscopic examination of muscle tissue samples obtained from the group that did not receive any treatment after I-R injury and were sacrificed 1 month later, peripheral heterochromatin increase was observed in the nuclei of muscle fibers. It was observed that the sarcomere structures were mostly in normal structure, however, some degenerative changes occurred in the cytoplasmic organelles. It was noted that the mitochondria located under the sarcolemma and between the myofibrils expanded from place to place and the cristae structures were disrupted. In addition, it was observed that the sarcoplasmic reticulum cisterns were enlarged and vacuoles containing membranous whorl structures of different sizes were formed in the cytoplasm (Figure 12).

Group 3 (PEMF-1 hour group): In electron microscopic examination of muscle tissue samples obtained from the group that

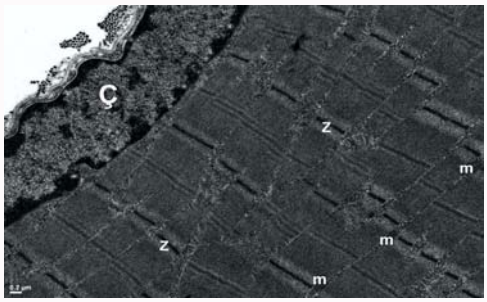

Figure 13: Electron microscopic view of the striated muscle of Group 3. The nucleus (C), mitochondria (m) and sarcomeres between two adjacent Z lines are observed in normal structure. Bar: 0.2 µm.

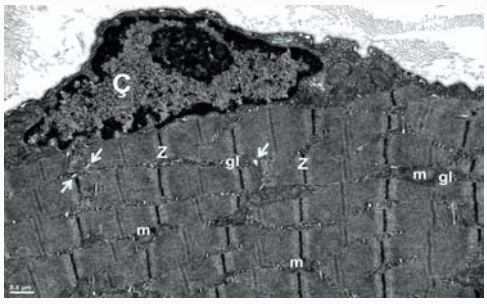

Figure 14: Electron microscopic view of the striated muscle of Group 4. The core of the muscle fiber (C), mitochondria (m), sarcoplasmic reticulum cisterns (arrow) and sarcomeres, which are contractile units located between the two Z lines, are observed in the normal structure. Glycogen particles (gl) are shown between the myofibrils. Bar: 0.5 µm.

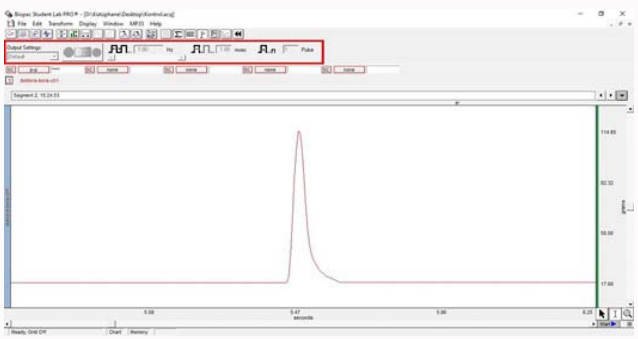

Figure 15: A contraction recording image of Group 1.

was treated with PEMF for 1 h a day for 1 month after I-R injury and sacrificed at the end of 1 month, it was observed that degenerative changes in the nucleus and cytoplasmic organelles of muscle fibers were partially reduced when compared to Group 2. Mitochondria, sarcoplasmic reticulum cisterns and sarcomeres between two adjacent Z lines were observed in the cytoplasm in normal structure (Figure 13).

Group 4 (PEMF-2 hours group): In electron microscopic examination of muscle tissue samples obtained from the group that was treated with PEMF for 2 h a day for 1 month after I-R injury and were sacrificed at the end of 1 month, it was observed that the nucleus, mitochondria, sarcoplasmic reticulum cisterns and glycogen particles between myofibrils were in normal structure. It was noted that the sarcomere structure and cytoplasm had a normal appearance, and the degenerative changes in the muscle fibers were reduced. It

Table 2: PT, RT and CT values measured in isolated organ bath.

Groups		PT	CT	RT
Group 1	1	9,48,396	35	95
	2	10,04,975	30	95
	3	10,01,053	35	105
	4	9,17,065	35	85
	5	10,05,813	30	85
	6	9,25,741	30	85
	7	9,50,426	35	85
	8	9,97,513	30	85
Group 2	1	5,94,293	35	95
	2	6,08,674	35	85
	3	7,05,461	30	95
	4	6,88,769	30	80
	5	6,06,695	35	95
	6	6,18,472	30	95
	7	6,03,347	45	95
	8	6,22,394	35	95
Group 3	1	8,04,428	35	95
	2	7,74,164	35	95
	3	7,98,844	40	95
	4	79,455	40	95
	5	7,57,623	40	90
	6	7,22,312	35	85
	7	7,84,078	40	90
	8	7,72,483	35	95
Group 4	1	10,05,426	30	90
	2	9,51,164	35	80
	3	95,897	35	80
	4	9,26,483	35	90
	5	91,385	35	95
	6	9,45,632	30	80
	7	9,39,401	35	90
	8	9,25,888	35	85

was observed that the organization of myofibrils in the muscle fibers was more regular compared to Group 2 and Group 3 and had similar characteristics with the control group (Figure 14).

Biophysical findings

Contraction force (PT, g), Contraction Time (CT, ms) and Relaxation Time (RT, ms) were evaluated in isometric muscle measurements made in the isolated organ bath (Table 2 and Figure 15).

According to the findings, the isometric single jolt contraction forces recorded from the gastrocnemius muscles of the rats in Group 1 were found to be greater than those of the other groups (Table 3). Compared to Group 1, 34.87% ($p<0.05$) strength reduction was observed in the gastrocnemius muscles of the rats in Group 2. When compared to Group 2, when the ratios of PEMF application increase isometric contraction forces; Group 3, 22.98%+ and Group 4, 49.89%+ muscle strength increase was observed. These results showed that 2-h

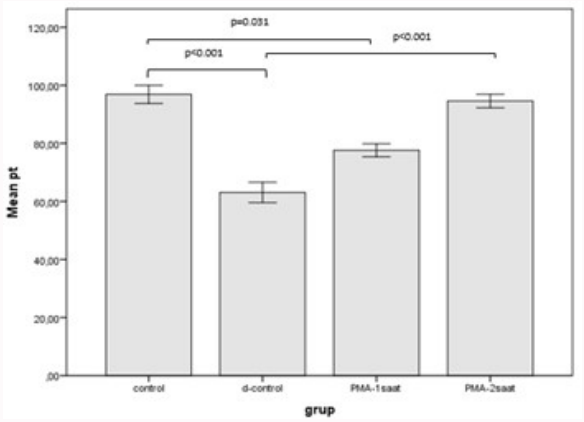

Figure 16: PT distribution chart by groups.

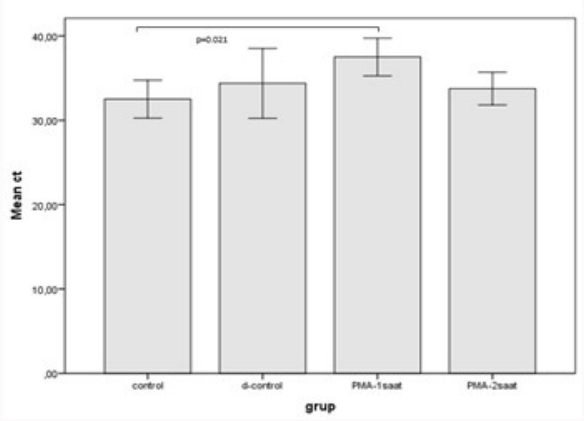

Figure 17: CT distribution chart by groups.

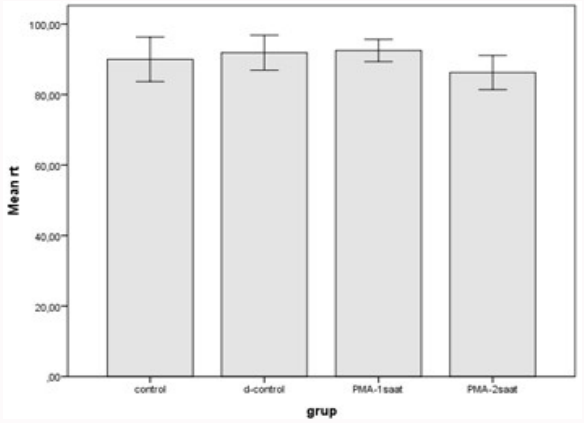

Figure 18: RT distribution chart by groups.

PEMF application was significantly ($p<0.05$) more effective than 1-h PEMF application. Compared to Group 1, it was observed that the contraction times recorded from the gastrocnemius muscles of the rats in Group 2, 3 and 4 were prolonged by 5.78%, 15.38% and 3.84%, respectively (Figure 16).

When examining the rates at which the response of prolonged contraction time with experimental ischemic atrophy decreased compared to Group 2 with the applied methods; it was observed that there was an increase of 9.09% in Group 3 and a decrease of 1.81%

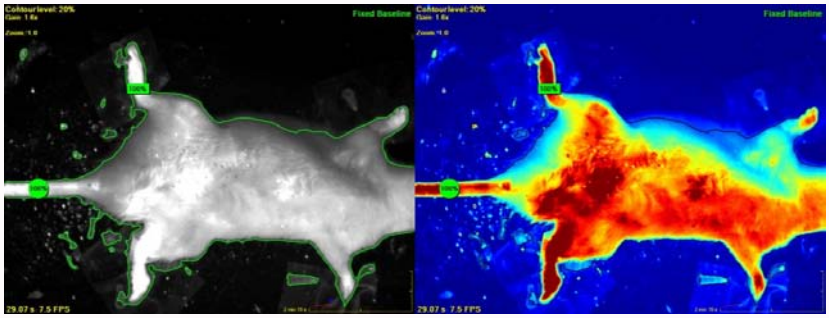

Figure 19: Perfusion Mapping Images of a Rat in Group 1.

Table 3: Descriptive statistics of clinical features.

	Groups				p value
	Group 1 (n=8)	Group 2 (n=8)	Group 3 (n=8)	Group 4 (n=8)	
Pt[a,b,c]	96.8 ± 3.7	63.1 ± 4.1	77.6 ± 2.66	94.5 ± 2.8	<0.001
	97.3 (91.7-100.5)	61.3 (59.4-70.5)	77.9 (72.2-80.4)	94.2 (91.3-100.5)	
Ct[b]	32.5 ± 2.7	34.3 ± 4.9	37.5 ± 2.7	33.7 ± 2.3	0.024
	32.5 (30.0-35.0)	35.0 (30.0-45.0)	37.5 (35.0-40.0)	35.0 (30.0-35.0)	
Rt	90.0 ± 7.5	91.8 ± 5.9	92.5 ± 3.7	86.2 ± 5.8	0.114
	85.0 (85.0-105.0)	95.0 (80.0-95.0)	95.0 (85.0-95.0)	87.5 (80.0-95.0)	

Data are summarized as mean ± standard deviation, median (min-max). a: p<0.05 Group 1 *vs.* Group 2, b: p<0.05 Group 1 *vs.* Group 3, c: p<0.05 Group 2 *vs.* Group 4

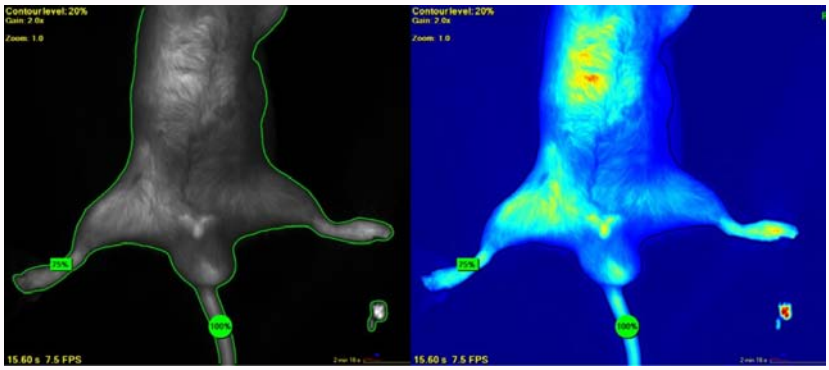

Figure 20: Perfusion Mapping Images of a Rat in Group 2.

in Group 4 (Figure 17). Compared to Group 1, the relaxation times recorded from the gastrocnemius muscles of the rats in Group 2 and 3 were longer by 2.08% and 2.77%, respectively, and shortened by 4.16% compared to the rats in Group 4. In Group 2, with the applied methods, the prolonged relaxation time responses with experimental ischemic atrophy; it was observed that it decreased by 0.68% from Group 3 and 6.12% from Group 4 (Figure 18).

According to the findings obtained from biomechanical recordings, it was observed that there were deteriorations in contraction forces, contraction times and relaxation times in gastrocnemius muscle atrophy induced by experimental ischemic injury. It was concluded that these deteriorations, which occur with atrophy, are best improved with 2 h of PEMF application.

Infrared fluorescent angiography perfusion evaluation findings

After ICG application from the tail vein, imaging was performed with the SPY device. While performing perfusion mapping, 100% of the tail vein where ICG was applied was taken as a basis. In the mapping in Group 1, the lower extremity perfusion was measured as 100%, which was blind to the tail vein (Figure 19). As a result of the perfusion evaluation performed with the SPY device, a decrease in perfusion mapping was observed in the lower extremities of the rats in Group 2 with I-R damage (Figure 20). It was seen that the perfusion was similar to each other in the images in Group 3 and Group 4, and had almost the same percentages as in Group 1 in the percentile analysis (Figure 21, 22).

Discussion

We encounter I-R in plastic surgery practice, as a result of tourniquet applications, clamps applied during surgery, separation of the flap from the donor area during free flap surgery, amputations, and arterial thrombi. Operations requiring microsurgery, such as free flap surgery, transplantations, and replantation's, involve a mandatory ischemia period of 1 h to 4 h until reperfusion with anastomosis is achieved. Especially in surgeries involving skeletal muscle such as free muscle flaps and replantation's, the ischemia time should be shortened in order not to lose the morphological and

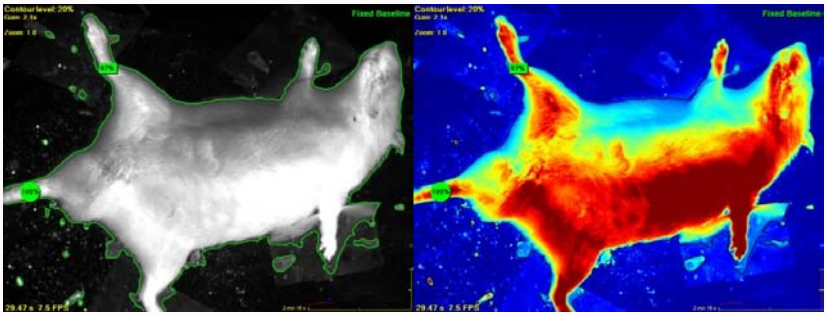

Figure 21: Perfusion Mapping Images of a Rat in Group 3.

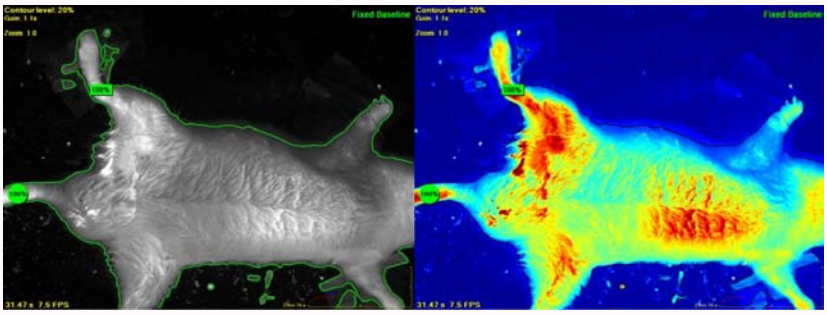

Figure 22: Perfusion Mapping Images of a Rat in Group 4.

functional properties of the muscle tissue, and measures should be taken to reduce the damage caused by reperfusion. Otherwise, it may result in a wide range of morbidities, such as limb loss, myocardial damage, acute renal and respiratory failure, and MODS, and even mortality [2].

Various surgical treatments can be applied to prevent I-R damage. Examples of these are embolectomies, ischemic preconditioning, controlled reperfusion, and flap delay procedures. Since these methods create an additional operation requirement, hospitalization times are prolonged, costs increase, and lead to time loss. Therefore, non-surgical, low-cost, easily accessible and applicable pharmacological treatments have been focused on.

The effects of antioxidant substances and agents that will activate the antioxidant mechanism have been studied in order to prevent this pathophysiological process or to reduce the damage it will cause. Melatonin [3], Deferoxamine [4], Vitamin C [5], Vitamin E [6], Selenium [7], Coenzyme Q [8], N-acetylcysteine [9,10], SOD [11], NOS inhibitors [12], L-arginine [13], NO precursors [14], thromboxane synthetase inhibitors [15], applications to these studies can be given as an example. In addition, nonsteroidal anti-inflammatory drugs that inhibit the COX pathway [16-18], agents that reduce cytokine formation [19], and complement system inhibitors [2,20,21], have been used. In the late period of I-R injury, a circulatory disturbance is observed due to the development of vasospasm as a result of smooth muscle dysfunction. In order to control this process, prostaglandin E1 [22], carvedilol [23], sildenafil [24], nitroglycerin [25], thrombolytic agents tissue Plasminogen Activator (tPA) [26], urokinase [27], streptokinase [28], and anticoagulant agents like heparin [29], low molecular weight heparin and aspirin [30], were used.

Today, the effects of magnetic fields on bone healing have received strong scientific support and widespread clinical acceptance [31-35]. After the positive effects of the magnetic field on bone healing were observed, its effects on other tissues and their mechanisms of action were the subject of study. There have been studies showing that it accelerates cartilage healing by increasing proteoglycan synthesis and inducing chondroblast differentiation [36], increasing collagen synthesis [37]. Its use in plastic surgery field is also had shown [38-40]. Taylor et al. [41], studied the effects of pulsed electromagnetic field on the postoperative pain in breast reduction patients. Some surgeons routinely used PEMF as a component of postoperative treatment when injecting fat into the face to increase fat graft survival. Itoh et al. showed that PEMF treatment accelerates pressure wound healing. Roland et al. [42], showed that the electromagnetic field stimulates neovascularization in the rat model. Weber and his colleagues conducted a study showing that pulsed electromagnetic field increased the viability of the rat groin composite flap [43].

The synthesis of growth factors (such as TGF, FGF) is mediated by Ca+2/Calmodulin dependent proteins. This system is activated by voltage-dependent Ca+2 channels. PEMF leads to depolarization in the cell membrane and provides regulation of Na+ and Ca+2 ion channels. Activation of these channels causes activation of second messenger pathways and gene expression. In their study, Markov et al. showed that PEMF increased the binding of Ca2+ to calmodulin, thus increasing the synthesis of growth factors [44]. Aaron et al. [45], showed that PEMF increased the production of Transforming Growth Factor-β (TGF-β) at the cellular level. Tepper et al. in the study of his colleagues, it was reported that PEMF significantly increased endothelial cell tubulization and proliferation as well as Fibroblast Growth Factor-2 (FGF-2) *in vitro* [46].

Many studies are showing that PEMF increases blood circulation in the area where it is applied. It affects vascular smooth muscle tone and provides vasodilation with Ca+2 ion regulation [47]. PEMF has been shown to increase neovascularization by increasing the expression of FGF-2 and FGFR1 [48]. In our study, the increase in capillary proliferation was examined under the light microscope as

an indicator of neovascularization, and Group 1 was taken as the baseline value. When compared to Group 2, it was observed that capillary proliferation increased in Groups 3 and 4, and this increase was more in Group 4 compared to Group 3.

After ischemia in the skeletal muscle, damage occurs with the effect of reperfusion. This damage is related to the destructive effect of inflammatory systems, microvascular changes, and cell death. Even if these effects disappear completely, skeletal muscle function can never be restored [49]. In our study, isometric contractions of the gastrocnemius muscle were compared to examine the effects of PEMF on the loss of skeletal muscle function due to I-R damage. There was a statistically significant difference between the groups in the contraction strength ($p<0.01$). In the comparisons of the groups with each other; a statistically significant difference was found between Group 1 and Group 2 ($p<0.05$), which proves that a successful I-R injury was created. There was a statistically significant difference between Group 2 and Group 3 and between Group 1 and Group 3 ($p<0.05$). These data show us that 1-h PEMF application improves the affected muscle function after I-R injury, but functional recovery is not at the level of normal muscle function. No statistically significant difference was observed between Group 1 and Group 4. This data shows that 2 h of PEMF application brings the function of a muscle with I-R damage so close to normal muscle function that there is no significant difference. When the contraction times were compared, a significant difference was observed between the groups ($p<0.024$), and the difference between Group 1 and Group 3 was the difference. There was no statistically significant difference between the other groups in terms of contraction times. When the relaxation times were examined, no statistically significant difference was observed between the groups ($p=0.114$). In the light of all these data, it can be said that PEMF treatment is effective in reversing the loss of function in the skeletal muscle after I-R injury, a return to normal cannot be achieved with 1 h of application, but functions return to near normal values after 2 h of application.

It has been reported that PEMF does not make muscle or bone tissue stronger than normal tissue, but accelerates the damaged tissue to reach its maximum strength. That is, the effects are at the cellular level, mainly through modulation of expected cytokine and growth factor release at all stages of repair. In this way, it accelerates the repair but does not cause abnormal repair of the wound [50]. The values obtained as a result of the isometric muscle strength measurements we made in our study showed that the contraction strength in the PEMF treatment group approached the control group but did not exceed this value.

In our studies, H&E staining was performed under the light microscope, and specific stains and immunohistochemical studies can be performed to better show the inflammatory process and tissue damage in future studies. The mechanism of action of PEMF also changes at the genetic level, and its effects can be monitored by examining apoptotic and antiapoptotic genes (Bcl-2, caspase, HSP-90) in future studies.

In the treatment of I-R injury, agents for certain steps of the mechanism have been tried, and none of them have found a place in the treatment. This situation has led to the use of agents that affect more than one mechanism, not a single mechanism. Considering the mechanisms of I-R injury, PEMF treatment is in an important position both in terms of having an effect on many steps in the development process and using its regeneration effects in the period after the damage has occurred. In addition, the fact that the treatment can be applied locally and does not cause systemic side effects is an advantage compared to other treatment options.

Conclusion

There are very limited studies examining the effects of PEMF treatment on skeletal muscle I-R injury, and there is no study in the literature examining the effects of I-R injury on dysfunction. This study is the first one showing the effects of PEMF on muscle dysfunction after I-R injury.

We think that PEMF treatment can be among the plastic, reconstructive, and aesthetic surgery treatment algorithms, considering the absence of systemic side effects and its effects on both the damage process and the subsequent healing process.

References

1. Siemionow M, Arslan E. Ischemia/reperfusion injury: A review in relation to free tissue transfers. Microsurgery. 2004;24(6):468-75.
2. Khalil AA, Aziz FA, Hall JC. Reperfusion injury. Plast Reconstr Surg. 2006;117(3):1024-33.
3. Jassem W, Roake J. The molecular and cellular basis of reperfusion injury following organ transplantation. Transplant Rev. 1998;12(1):14-33.
4. Carden DL, Granger DN. Pathophysiology of ischemia-reperfusion injury. J Pathol. 2000;190(3):255-66.
5. Collard CD, Gelman S. Pathophysiology, clinical manifestations, and prevention of ischemia-reperfusion injury. Anesthesiology. 2001;94(6):1133-8.
6. Patel KD, Zimmerman GA, Prescott SM, McEver RP, McIntyre TM. Oxygen radicals induce human endothelial cells to express GMP-140 and bind neutrophils. J Cell Biol. 1991;112(4):749-59.
7. Kokura S, Yoshida N, Yoshikawa T. Anoxia/reoxygenation-induced leukocyte-endothelial cell interactions. Free Radic Biol Med. 2002;33(4):427-32.
8. Serizawa A, Nakamura S, Suzuki S, Baba S, Nakano M. Involvement of platelet-activating factor in cytokine production and neutrophil activation after hepatic ischemia-reperfusion. Hepatology. 1996;23(6):1656-63.
9. Chensue SW, Remick DG, Shmyr-Forsch C, Beals TF, Kunkel SL. Immunohistochemical demonstration of cytoplasmic and membrane-associated tumor necrosis factor in murine macrophages. Am J Pathol. 1988;133(3):564-72.
10. Frangogiannis NG, Lindsey ML, Michael LH, Youker KA, Bressler RB, Mendoza LH, et al. Resident cardiac mast cells degranulate and release preformed TNF-α, initiating the cytokine cascade in experimental canine myocardial ischemia/reperfusion. Circulation. 1998;98(7):699-710.
11. Ikeda U, Ikeda M, Kano S, Shimada K. Neutrophil adherence to rat cardiac myocyte by proinflammatory cytokines. J Cardiovasc Pharmacol. 1994;23(4):647-52.
12. Otamiri T, Lindahl M, Tagesson C. Phospholipase A2 inhibition prevents mucosal damage associated with small intestinal ischaemia in rats. Gut. 1988;29(4):489-94.
13. Weiss SJ. Tissue destruction by neutrophils. N Engl J Med. 1989;320(6):365-76.
14. Bagge U, Amundson B, Lauritzen C. White blood cell deformability and plugging of skeletal muscle capillaries in hemorrhagic shock. Acta Physiol Scand. 1980;108(2):159-63.
15. Carden DL, Smith JK, Korthuis RJ. Neutrophil-mediated microvascular dysfunction in postischemic canine skeletal muscle. Role of granulocyte adherence. Circ Res. 1990;66(5):1436-44.

16. Jassem W, Fuggle SV, Rela M, Koo DDH, Heaton ND. The role of mitochondria in ischemia/reperfusion injury. Transplantation. 2002;73(4):493-9.

17. Ondiveeran H, Fox-Robichaud A. New developments in the treatment of ischemia/reperfusion injury. Curr Opin Investig Drugs. 2001;2(6):783-91.

18. Ulugöl A, Karadağ CH, Dökmeci D, Gündüz O, Topuz RD. Farmakoloji. 1st Ed. Nobel Tıp Kitabevleri; 2017.

19. Yilmaz O. Dişi Reprodüktif Sistemde Yeni Bir Nörotransmitter: Nitrik Oksit. Kocatepe Vet J. 2010;3(2):65-70.

20. Smith GW, McArthur CJ, Simpson IJ. Circulating immune complexes in myocardial infarction. J Clin Lab Immunol. 1983;12(4):197-9.

21. Abbas AK. Basic immunology: Functions and disorders of the immune system, 6th Ed. J Chem Inf Model. 2017;110.

22. Hamann A, Andrew DP, Jablonski-Westrich D, Holzmann B, Butcher EC. Role of alpha 4-integrins in lymphocyte homing to mucosal tissues *in vivo*. J Immunol. 1994;152(7):3282-93.

23. Krötz F, Sohn HY, Pohl U. Reactive oxygen species: Players in the platelet game. Vol. 24, Arterioscler Thromb Vasc Biol. 2004;24(11):1988-96.

24. Hensley K, Robinson KA, Gabbita SP, Salsman S, Floyd RA. Reactive oxygen species, cell signaling, and cell injury. Free Radic Biol Med. 2000;28(10):1456-62.

25. Kvietys PR, Granger DN. Endothelial cell monolayers as a tool for studying microvascular pathophysiology. Am J Physiol. 1997;273(6):G1189-99.

26. Kalogeris T, Baines CP, Krenz M, Korthuis RJ. Ischemia/Reperfusion. Compr Physiol. 2016;7(1):113-70.

27. Romanenko VG, Davies PF, Levitan I. Dual effect of fluid shear stress on volume-regulated anion current in bovine aortic endothelial cells. Am J Physiol Cell Physiol. 2002;282(4):C708-18.

28. Al-Mehdi AB, Zhao G, Fisher AB. ATP-independent membrane depolarization with ischemia in the oxygen-ventilated isolated rat lung. Am J Respir Cell Mol Biol. 1998;18(5):653-61.

29. Kelsall CJ, Brown MD, Hudlicka O. Alterations in reactivity of small arterioles in rat skeletal muscle as a result of chronic ischaemia. J Vasc Res. 2001;38(3):212-8.

30. Fruchterman TM, Spain DA, Wilson MA, Harris PD, Garrison RN. Complement inhibition prevents gut ischemia and endothelial cell dysfunction after hemorrhage/resuscitation. Surgery. 1998;124(4):782-91.

31. Tiefenbacher CP, Chilian WM, Mitchell M, DeFily DV. Restoration of endothelium-dependent vasodilation after reperfusion injury by tetrahydrobiopterin. Circulation. 1996;94(6):1423-9.

32. Iràculis E, Cequier A, Gómez-Hospital JA, Sabaté M, Mauri J, Fernández-Nofrerias E, et al. Early dysfunction and long-term improvement in endothelium-dependent vasodilation in the infarct-related artery after thrombolysis. J Am Coll Cardiol. 2002;40(2):257-65.

33. Horwitz LD, Kaufman D, Keller MW, Kong Y. Time course of coronary endothelial healing after injury due to ischemia and reperfusion. Circulation. 1994;90(5):2439-47.

34. Alexander JS, Alexander BC, Eppihimer LA, Goodyear N, Haque R, Davis CP, et al. Inflammatory mediators induce sequestration of VE-cadherin in cultured human endothelial cells. Inflammation. 2000;24(2):99-113.

35. Gute DC, Ishida T, Yarimizu K, Korthuis RJ. Inflammatory responses to ischemia and reperfusion in skeletal muscle. Mol Cell Biochem. 1998;179(1-2):169-87.

36. Norton LA, Rovetti LA. Calcium incorporation in cultured chondroblasts perturbed by an electromagnetic field. J Orthop Res. 1988;6(4):559-66.

37. Farndale RW, Murray JC. Pulsed electromagnetic fields promote collagen production in bone marrow fibroblasts *via* a thermal mechanisms. Calcif Tissue Int. 1985;37(2):178-82.

38. Strauch B, Herman C, Dabb R, Ignarro LJ, Pilla AA. Evidence-based use of pulsed electromagnetic field therapy in clinical plastic surgery. Aesthet Surg J. 2009;29(2):135-43.

39. Kinney B. Pulsed electromagnetic field therapy in plastic surgery. Aesthet Surg J. 2005;25(1):87-91.

40. Henry SL, Concannon MJ, Yee GJ. The effect of magnetic fields on wound healing. Eplasty. 2008;8:e40.

41. Taylor E, Hardy K, Alonso A, Pilla A, Rohde C. Pulsed electromagnetic fields dosing impacts postoperative pain in breast reduction patients. J Surg Res. 2015;193(1):504-10.

42. Roland D, Ferder M, Kothuru R, Faierman T, Strauch B. Effects of pulsed magnetic energy on a microsurgically transferred vessel. Plast Reconstr Surg. 2000;105(4):1371-4.

43. Weber RV, Navarro A, Wu JK, Yu H-L, Strauch B. Pulsed magnetic fields applied to a transferred arterial loop support the rat groin composite flap. Plast Reconstr Surg. 2004;114(5):1185-9.

44. Markov MS, Pilla AA. Weak static magnetic field modulation of myosin phosphorylation in a cell-free preparation: Calcium dependence. Bioelectrochem Bioenerg. 1997;43(2):233-8.

45. Aaron RK, Wang S, Ciombor DMK. Upregulation of basal TGFβ1 levels by EMF coincident with chondrogenesis - Implications for skeletal repair and tissue engineering. J Orthop Res. 2002;20(2):233-40.

46. Ames 3rd A, Wright RL, Kowada M, Thurston JM, Majno G. Cerebral ischemia. II. The no-reflow phenomenon. Am J Pathol. 1968;52(2):437-53.

47. Gurtner GC, Neligan PC, Warren RJ, van Beek A. Plastic surgery: Principles. 3rd Ed. Elsevier Saunders; 2012.

48. Cunha MS, da Silva JCF, Nakamoto HA, Ferreira MC. Study of warm ischemia followed by reperfusion on a lower limb model in rats: Effect of allopurinol and streptokinase. Clinics (São Paulo). 2005;60(3):213-20.

49. Bihari A, Cepinskas G, Forbes TL, Potter RF, Lawendy AR. Systemic application of carbon monoxide-releasing molecule 3 protects skeletal muscle from ischemia-reperfusion injury. J Vasc Surg. 2017;66(6):1864-71.

50. Rosero O, Németh K, Turóczi Z, Fülöp A, Garbaisz D, Győrffy A, et al. Collateral circulation of the rat lower limb and its significance in ischemia–reperfusion studies. Surg Today. 2014;44(12):2345-53.