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Ischemia- Reperfusion Challenge in Human Skeletal Muscle: Study in Knee Arthroplasty Surgery

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Abstract

Background: There are many studies that analyze ischemia-reperfusion injury in different organs (brain, heart...) but they are very scarce in skeletal muscle. The few studies performed in humans show partial results, and none include morphological, inflammatory, biochemical, metabolic and hemodynamic alterations.

The objective of our study was to typify ischemic and ischemia-reperfusion injury in skeletal muscle in patients undergoing total knee arthroplasty from several points of view: morphological, biochemical, metabolic and hemodynamical, as well as to evaluate the protective effect of a single-dose of corticoids on the above-mentioned parameters.

Methods: Forty-one patients participated in the study. They were randomly assigned to either group A: no corticoid administered; or to group B: a single dose of methylprednisolone was administered before performing ischemia. Blood samples were drawn at different intervals for determination of inflammatory biomarkers and necrosis enzymes, and three muscle biopsies were performed for histological studies.

Results: The ischemia period ranged from 50 to 84 minutes in both groups. There were no histological differences either between the groups. We found significant differences in inflammatory markers: there was a higher increase in C-reactive protein, erythrocyte sedimentation rate and Interleukin-6 in group A, while the leukocyte count was higher in group B.

Conclusions: Human skeletal muscle can bear periods of ischemia followed by reperfusion as far as 1.5 h without showing any sign of structural, ultrastructural or inmunoshistochemical damage. We have described the biochemical changes that take place in patients submitted to total knee arthroplasty with tourniquet under spinal anesthesia during the first 48 h post-procedure. Adding a single dose of methylprednisolone 7 mg/kg IV immediately prior to surgery significantly reduced the levels of some markers of morbidity.

Keywords: Ischemia; Reperfusion; Skeletal muscle; Knee; Arthroplasty; Corticoids

Introduction

The use of a tourniquet to achieve ischemia is a common practice in orthopedic surgery of upper and lower limbs. It allows a better view of anatomic structures in the surgical field making dissection easier, reducing intraoperative bleeding and providing a better cement-bone interface in a cemented arthroplasty [1]. However, reported complications include muscle, nerve and vascular damage secondary to ischemia-reperfusion, and potential cardiorespiratory complications in patients with poor basal cardiac status. Some authors report a greater risk of deep-venous thrombosis, increased incidence of wound infections and more postoperative pain [2,3]. Cardiac arrest or severe hemodynamic instability immediately after release of tourniquet are rare but observed complications.

Any organ or tissue undergoing prolonged ischemia followed by reperfusion can suffer reversible or irreversible damage, in which pathophysiological mechanisms related to the ischemia and to the following reperfusion are involved [4]. There are many studies that analyze the damage induced by ischemia-reperfusion in many organs: heart, liver, nervous system, kidneys, gut [5-18] in human and animal models. Few are focused on skeletal muscle, and most of these are in vitro studies related to animal models [19-22]. Studies performed in vivo in human skeletal muscle are very scarce, and none include morphological, inflammatory, biochemical, metabolic and hemodynamic changes. None study the possible protective effect of corticoids in ischemia-reperfusion injury in skeletal muscle after the use of a tourniquet.

The major aim of the study was to increase our knowledge about in vivo changes in human skeletal muscle secondary to ischemia-reperfusion.

We chose total knee arthroplasty (TKA) as the object of our study since we considered that it comprises a quite uniform study group and it reproduces well the ischemia-reperfusion conditions. Moreover, the use of a tourniquet is an accepted standard in this surgery. The use of corticosteroids was limited to a single dose. Diabetic patients were excluded from the study to avoid greater fluctuations in glucemia. The administration of corticosteroids was based on multiple studies [23-29] performed in cardiac, aortic and major abdominal surgery that demonstrated an inhibition of systemic inflammatory response, and a decrease of certain biomarkers of morbidity (CRP, IL-6).

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The aims of our study were:

1. To define ischemic and ischemia-reperfusion damage in patients undergoing TKA from several points of view:

a) morphological (structural, ultrastructural, and immunohistochemical).

b) biochemical (necrosis enzymes, cytokines, inflammatory biomarkers).

c) metabolic (lactic acid, glucose, pH and ions).

2. To evaluate the protective effect of a single dose of corticosteroid (7 mg/kg of methylprednisolone IV) on the above-mentioned parameters.

Material and Methods

Written, informed consent was obtained from each patient after the study was approved by the Investigation Committee and the Clinical Research Ethics Committee of the Hospital General Universitario Gregorio Marañon.

The study was designed as a pilot clinical trial, with randomized and controlled parallel groups. Sample size was not predetermined because the study was meant as a pilot study, and about 20 patients were recruited for each group.

Inclusion criteria were: Adult ASA I and II patients undergoing TKA. Exclusion criteria were: ASA III or IV, diabetic patients and patients on chronic corticoid treatment.

Patients were randomly assigned (by tables of random numbers) to one of two groups:

Group A: patients that did not receive corticosteroids

Group B: patients receiving a single dose of 7 mg/kg of methylprednisolone prior to initiation of ischemia.

All patients underwent spinal anesthesia (L3-L4) with hyperbaric bupivacaine 0.5% (11-13 mg) and were sedated with diazepam (2-8 mg IV). They were monitored by EKG, invasive blood pressure (radial artery) and pulse oximetry. All of them received antibiotic prophylaxis with 2 g cephazoline IV. Leg ischemia was produced after member exsanguinations (Esmarch) by tourniquet inflation to 300 mm Hg.

Evaluated variables

Blood samples were drawn at specified times: a) basal, upon arrival to the operating room; b) 5 minutes after ischemia; and c, d, e) at 4, 24 and 48 hours post-ischemia and were sent immediately to the Laboratory Department.

The evaluated variables were:

Primary variables: leukocyte count (granulocyte %), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor (TNF) and creatine phosphokinase (CPK).

Secondary variables: demographic data (age, sex, weight, height); personal background (associated diseases, chronic medication); surgical data (duration of surgery, duration of ischemia); hemodynamic data (heart rate, systolic and diastolic blood pressure, oxygen saturation); other laboratory data (lymphocyte %, monocyte %, hemoglobin, hematocrit, platelet count, lactic acid, transaminases (GOT, GPT), lactic dehydrogenase (LDH), sodium, potassium, glycemia, pH and bicarbonate.

During the study, 3 muscular biopsies were taken from the vastus lateralis exposed in the surgical field. The first was taken upon surgical incision right after initiating ischemia. The second, before deflating the tourniquet, thus before reperfusion. And the third, 15 min after reperfusion. The specimens were impregnated with talcum powder, introduced in a dry plastic bottle, and sent immediately to the Pathology Department. They were then frozen in liquid nitrogen and stained. The usual process consisted of: conventional staining with hematoxylin and eosin, Gomori's trichromic stain, and staining for succinate-dehydrogenase (SDH), adenosine triphosphatase (ATPase), cytochrome oxidase and NADH tetrazolium reductase. Immunohistochemic studies were also performed for HLA 1, CD68, CD45, CD4, CD8, perforin, β-chrystallin and dystrophin 1.

Statistical analysis

Data was presented as mean ± SEM, and repeated measures analysis of variance (ANOVA) was performed.

To determine the basal homogeneity of both groups, we used Student's t test for independent variables, and Fischer's exact test for categorical variables. Statistical significance was considered for $p<0.05$.

Statistical analysis was performed with Windows SPSS, version 12.0

Results

In group A, one patient was excluded because during the study he developed hepatitis of unknown ethiology (with hypertransaminasemia), and 2 patients were excluded due to loss of data, so we finally had 17 patients in this group. Group B, (corticosteroid group) comprised 21 patients. (Figure 1)

Homogeinity of the two groups

The demographic data and surgical and ischemic times of the 38 patients are summarized in table 1. There are no statistically significant differences.

Primary variables

Leukocyte count (LC) (granulocyte %): In both groups, we observed an increase in LC, reaching its highest value after 4 hours.

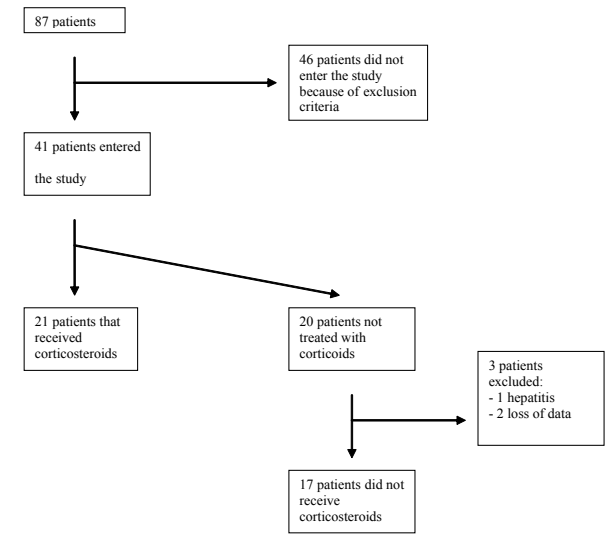
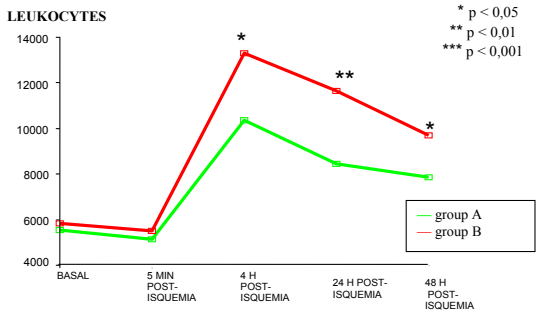


Figure 1: Flow of patients.

	Group A	Group B	p
Age (yr)	71,88 ± 1,36	72,19 ± 1,61	0,888
Height (cm)	156,73 ± 1,23	160,31 ± 1,98	0,136
Weight (kg)	76,58 ± 2,75	75,85 ± 2,13	0,835
Ischemia time (min)	62,85 ± 5,53	64,15 ± 2,93	0,825
Surgical time (min)	75 ± 7,95	90,31 ± 4,62	0,103

Table 1: Demographic data and duration of surgery and ischemia.



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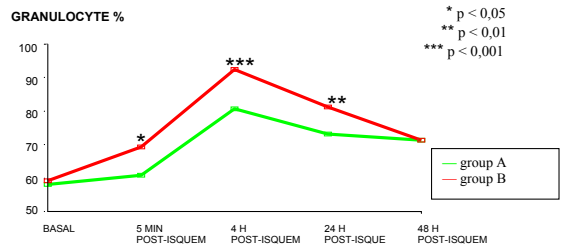


Figure 3:

In general, the increase in LC in group A was smaller than in group B ($p<0.05$). This same pattern is also seen in the granulocyte count. (figure2, 3)

CRP: Both groups showed an increase in CRP beginning at 4h and remaining elevated for at least 48 hours, although the increase was smaller in the corticosteroid group ($p<0.001$) (Figure 4)

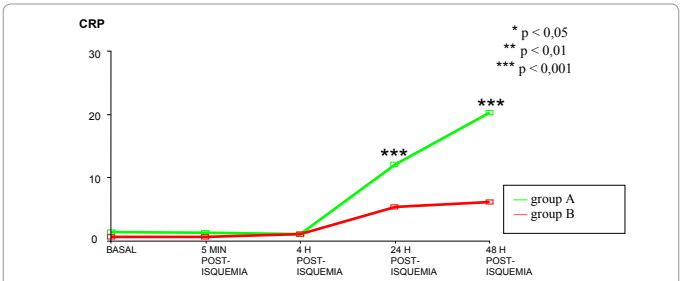
ESR: In both groups, ESR increased after the first 4 h and continued increasing for at least 48 hours post-ischemia Again, the increase was smaller in the corticosteroid group ($p<0.05$) (Figure 5).

IL-6: There is also an increase in IL-6, but this elevation is observed sooner than with the previous parameters. IL-6 starts to increase at 5 min post-ischemia and reaches its peak value at 24 hours. The elevation in IL-6 is less ($p<0.05$) in the corticosteroid group (Figure 6).

TNF: There was an early rise of TNF, reaching its peak at 4 hours, but there were no significant differences between both groups (Figure 7).

CPK: Creatine phosphokinase levels showed a tendency to increase, reaching its maximum after 24 hours, but the difference between both groups was not significant (Figure 8).

Others: We observed marked acidosis 24 hours after ischemia related to a significant lactic acid increase with similar results in both groups. We did not find changes in values of GOT, GPT, LDH, sodium, potassium and glucose.



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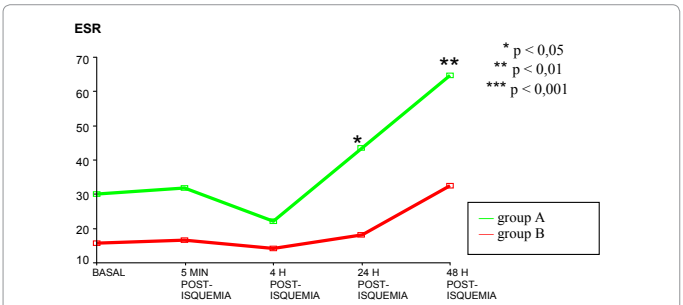

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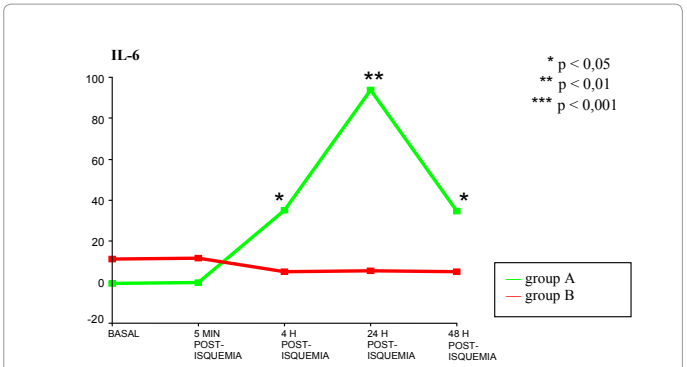

Figure 6:

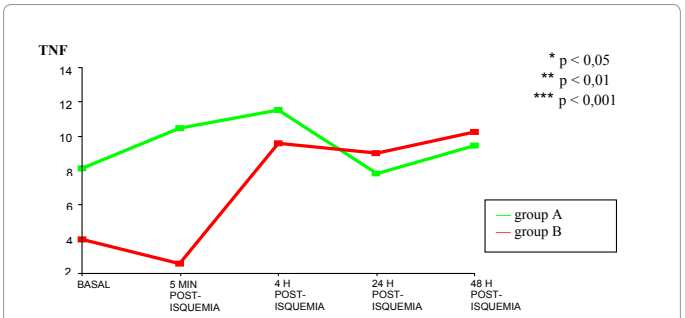

Figure 7:

Anatomopathological studies

In the biopsy specimens of our study, there were no structural changes secondary to ischemia and/or reperfusion. We did not find

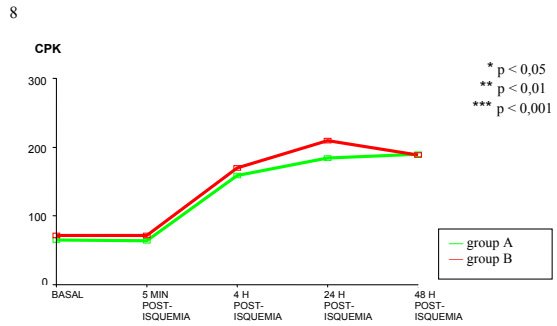

Figure 8:

necrosis, destructuring of the oxidative pattern, inflammatory signs (edema, polimorphonuclear or histiocyte pooling) nor vascular changes (endothelial damage, vascular thrombosis) in any of the samples.

Histological findings are very similar in all the biopsies taken before surgery: several atrophied type II fibers secondary to disuse, with no accumulation of inflammatory cells.

Discussion

On Material and methods

Total knee arthroplasty is a reliable and appropriate procedure for moderate to severe knee arthrosis in 50-year old patients and older. We chose this surgical procedure because it is done in a more or less similar age population (in our case, the mean age was 72 years). We selected ASA I and II patients (excluding diabetics) to make the study groups more homogeneous.

The operation was performed by the same group of orthopedic surgeons to avoid great fluctuations in surgical times (and thus, ischemia times) between patients.

Corticoid administration

Several studies report that preoperative administration of corticoids (methylprednisolone 30 mg/kg) attenuate the systemic inflammatory response syndrome in patients undergoing heart surgery with extracorporeal circulation [8] or hepatic surgery [30,31]. It inhibits cellular and plasmatic inflammatory responses, reduces Complement-mediated neutrophil activation, decreases proinflammatory/antiinflammatory interleukin ratio, decreases CRP levels and minimizes tissular responses [24,25,29,32-34]. Based on these findings, preoperative administration of corticoids is a routine practice in many hospitals to attenuate the inflammatory response after surgical aggression.

In our study, we administered a single dose of methylprednisolone 7 mg/kg IV prior to ischemia, based upon previous studies in which similar doses were used for hepatic resection [35], abdominal aortic surgery [36] and cardiac surgery[37,38], obtaining in all of the cases a decrease of inflammatory markers.

On Results

Structural changes

Ischemia-reperfusion of skeletal muscle produces histological changes that consist of cellular edema and fatty degeneration when the injury is still reversible. Cellular edema is the first manifestation of almost all types of cellular damage and is a consequence of the passage of extracellular water into the cell. When water continues to accumulate inside the cell, small clear vacuoles appear in the cytoplasm. This is known as hydropic or vacuolar degeneration. If the damage becomes irreversible, necrosis of the cell is produced [39].

All samples were stained for HLA1, CD68, CD45RO, CD4, CD8, perforin, and β-chrystallin to show inflammatory changes, and stained with dystrophin to evaluate sarcolemmal damage. We found no definite morphological changes suggesting membrane injury or muscular inflammation in any of the samples, nor leukocyte infiltration or fiber necrosis. The short duration of ischemia in our study (an average of 63 minutes, maximum of 105 minutes) might explain why the anatomopathological changes were so scarce.

Biochemical changes

Leukocytes: There was a clear increase in leukocyte count, already detected in the sample taken after five minutes of ischemia. This increase was highest at 4 hours post-ischemia. The increase in leukocyte count was mainly due to granulocytes. This finding corresponds with what other authors such as Wakai et al demonstrated in their study of the inflammatory response in patients undergoing arthroscopy [40]. This leukocytosis, with a high proportion of granulocytes and few lymphocytes, is characteristically seen after surgery, and is related to the degree of trauma and duration of ischemia.

When comparing the two groups, we found a higher increase in leukocytes, especially granulocytes, in the patients who received corticoids. These results can be explained by the strong anti-inflammatory action of glucocorticoids, that produce a transitory increase of circulating neutrophils [41]. This is caused by the following mechanisms [42]:

a) Adherence of neutrophils to endothelium decreases, preventing its accumulation in the inflamed area and increasing its number in peripheral blood.

b) Polymorphonuclear leukocytes are released from the bone marrow

c) Half-life of neutrophils increases

Probably one of the most important mechanisms of the anti-inflammatory action of glucocorticoids is the inhibition of accumulation of neutrophils in the inflammated area [43].

Studies on the benefits of preoperative administration of corticoids show contradictory results with respect to hemodynamic changes, pulmonary function and glucose metabolism during the post-operative period. Clinical investigations by Chaney et al. [44] and Morariu et al. [45] show that preoperative administration of high doses of methyprednisolone do not offer clinical advantages, and may even be detrimental due to postsurgical hyperglycemia and unexplained late extubation.

In our study, we administered doses of up to 500 mg of methyprednisolone. There were no hemodynamic alterations, pulmonary complications or uncontrolled glycemia. There were no statistically significant differences between basal and 48-hour glucose levels.

C-reactive protein: This is a plasma protein first described by Tillet and Francis in 1930. It is mainly synthesized by the liver, although it can also be produced extrahepatically in neurons, arteriosclerotic plaques, monocytes and lymphocytes. Normal level in healthy people is less than 10 mg/dl, but it increases rapidly after an acute inflammatory stress. It

participates in systemic inflammatory responses and is important in maintaining homeostasis [46]. It activates Complement and increases phagocytosis. It also regulates molecular adhesion in endothelial cells and increases liberation of IL-1, IL-6 and TNF [47].

In our study, the pattern of the CRP elevation (which reached its maximum in 48 hours post-reperfusion) was similar to that seen by other authors in different studies. Orrego et al. [48], in a study on CRP levels after elective orthopedic surgery, and Larsson et al. [49] found an increase of CRP, which was highest at 48 hours. Larsson et al. compared hip arthroplasty, unicompartmental knee arthroplasty and lumbar micro-discectomy and found that the knee arthroplasty group presented the highest increase in CPR. They attributed this higher increase to the use of a tourniquet.

Erythrocyte Sedimentation Rate: Erythrocyte sedimentation rate (ESR) is a measure of the setting of red blood cells in a tube of unclotted blood (treated with sodium citrate) in a period of time (normally one hour). It is an indirect measure of the concentration of acute phase reactants in plasma. ESR is a less sensitive indicator than CRP [50], and its levels change more slowly than CRP when a patient's condition improves or worsens [51]. In our study we observed a significant increase of ESR at 24 hours post-op that remained elevated after 48 hours. According to different authors [49,52,53], ESR can remain elevated during months and even a year after uncomplicated surgery. Our study was limited to 48 hours, so we did not contrast this assertion. We did observe a lower increase of ESR at 24 and 48 hours postop in the group that received corticoids compared to the control group. These findings are consistent with those of several papers that study the effect of corticoids in rheumatic and endocrine diseases [54,55].

Interleukin-6: This is one of the best studied cytokines. It participates in the regulation of many immunologic reactions. The role of IL-6 in the acute phase response secondary to surgery has been well studied. Several authors suggest that IL-6 is the most precise inflammation and post-op acute phase response marker. Elective surgery causes an increase in IL-6 levels between 1 and 3 hours after the beginning of surgical aggression, and can remain elevated between 48 and 72 hours. The increase of IL-6 levels is directly related to the degree of tissue damage. IL-6 is considered a direct marker of postoperative morbi-mortality. There are several studies in cardiac and esophageal surgery that establish a relationship between elevated IL-6 levels and an increase in postoperative morbi-mortality56.

Like others [57] we found a significant increase in IL-6 levels at 4 hours postop, indicating an acute phase inflammatory response. This increase was much less pronounced in the group of patients receiving corticoids ($p<0.05$) and supports findings of other clinical studies, especially in cardiac surgery under extracorporeal circulation, that preoperative treatment with corticoids administered prior to ischemia reduces the liberation of pro-inflammatory cytokines [23,58,59]. It could be that glucocorticoid administration blocks the expression of IL-6 gene. Other authors attribute the lower increase of IL-6 to the fact that corticoids increase the synthesis of interleukin-10 (IL-10), an anti-inflammatory cytokine that inhibits the synthesis of IL-6 [60]. We did not measure IL-10 in our study.

Tumor necrosis factor: Tumoral necrosis factor belongs to the group of cytokines that stimulate the acute phase of an inflammatory reaction [61]. The local effects of TNF are beneficial, but when TNF operates systemically its effects can be deleterious and cause syndromes such as septic shock or disseminated intravascular coagulation. We found no statistically significant differences in TNF levels between both groups.

Creatine phosphokinase: During the study, we had in mind the findings caused by ischemia as well as those caused by reperfusion. Until recently, ischemic damage was thought to end when the tourniquet was released. Presently, we know that reperfusion is necessary to avoid irreversible damage, but it also can cause important injuries [62].

Creatine phosphokinase (CPK) is an enzyme that plays an important role in the energy metabolism of skeletal muscle. It catalyzes the reversible reaction: Phosphocreatine + ADP↔ Creatine + ATP. During active contraction, this reaction is towards synthesis of ATP, but during the recovery phase of exercise, the same enzyme is utilized to synthesize phosphocreatine from creatine and ATP [63].

The role of CPK during reperfusion has been studied in depth during the last few years. It seems that CPK activation has a protective role in muscle during ischemia-reperfusion [64].

In our study CPK levels increased and reached a peak at 24 hours. These findings are similar to those of Olivei et al. in their paper on muscular damage indices in peripheral revascularization procedures [65]. They correspond with what is well established in other studies, especially in acute myocardial infarction, where the peak of CPK elevation is observed at 18-24 hours of an ischemic event. We didn't find statistically significant differences between group A and group B. The levels of CPK were not very high since there was no muscular necrosis. For the same reason, hepatic enzymes and potassium levels hardly increased.

Up to our knowledge, there are few reports that investigate in vivo structural damage and systemic inflammatory response secondary to ischemia-reperfusion in the human skeletal muscle. The great majority of studies are performed in animal models, and the information obtained in these may not be totally relevant for humans due to species differences; for example, mice's skeletal muscle is more susceptible to ischemia than that of dogs [66,67]. According to our results, human skeletal muscle can bear periods of ischemia followed by reperfusion as far as 1.5 h without showing any sign of structural, ultrastructural or inmunohistochemical damage. We have described over the time the biochemical changes in patients submitted to TKA with tourniquet under spinal anesthesia. Adding a single dose of corticoids prior to surgery reduce very significantly the levels of markers of morbidity such as IL-6 or CRP. As far as we know, there are not studies that analyzed the action of corticoids in the response to ischemia reperfusion in human skeletal muscle *in vivo*.

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