Can Prolonged Ischemia Time in Extremity Transfer Be Resolved Using an Extracorporeal Circulation Model? An Experimental Study

Suleyman Yildizdal, MD,[a] Özbeyen Atalay, PhD,[b] Deniz Önal, PhD,[c] Bilge Pehlivanoğlu, MD, PhD,[b] Beril Talim, MD,[d] Özay Gököz, MD,[e] and Serdar N. Nasır, MD[f]

Background: The standard method for transporting tissues during limb transplantation and replantation is cold ischemic transport (CIT). However, CIT cannot completely prevent ischemia-reperfusion injury (IRI). As an alternative, extracorporeal perfusion (ECP) methods that provide an adequate metabolic environment for ischemic tissues could be considered. In this study, we investigated the differences between CIT and ECP in terms of their effects on IRI.

Methods: An ischemia-reperfusion model was used to compare the CIT and ECP groups. This model includes a 6-hour ischemia period followed by a one-hour reperfusion period. Superoxide dismutase, catalase, total antioxidant status, total oxidant status, and total thiol levels in muscle and blood samples were biochemically analyzed to determine oxidative damage levels. TNF-α, NF-κB, and IL-10 levels were measured in the same samples to evaluate the degree of inflammation. Apoptosis was evaluated by measuring the levels of Bax and Bcl-2 proteins in muscle samples. Histopathologic examination was performed for tissue damage and mitochondria were evaluated by Cox staining.

Results: It was found that the ECP causes less oxidative and inflammatory damage than the CIT. Bax and bcl-2 levels did not differ between the 2 groups. Biochemical parameters were found to be higher in the CIT group. More mitochondrial damage was observed in the CIT system.

Conclusions: ECP caused less inflammatory and oxidative damage compared with CIT. The promising results of our experimental study suggest that the clinical use of extracorporeal circulation machines for extremity transport may reduce histopathologic damage.

Key Words: ischemia-reperfusion injury, oxidative stress, extracorporeal circulation model, cold ischemic transport method

(*Ann Plast Surg* 2026;96:455–462)

Currently, cold ischemic transport (CIT) remains the standard method for extremity transport in replantation and allotransplantation because of its cost-effectiveness, accessibility, technical simplicity, and practical feasibility. However, despite being generally regarded as adequate for tissues with high metabolic demands or extremities containing large muscle mass, prolonged cold storage fails to sufficiently prevent ischemia-reperfusion injury (IRI). This inherent limitation is frequently associated with suboptimal functional recovery and decreased survival of replanted or transplanted extremities. Moreover, in cases involving multiple extremity amputations, the prolonged operative times inherent to CIT further extend ischemia duration, thereby exacerbating tissue injury and compromising clinical outcomes. In contrast, extracorporeal perfusion (ECP) techniques have demonstrated the potential to attenuate IRI, modulate systemic inflammatory responses, and offer meaningful advantages over conventional cold-storage methods.[1–4]

Previous studies have demonstrated that the preservation of vascularized composite tissues using ECP systems significantly reduces ischemic tissue injury.[1,2] These systems provide continuous oxygenation, maintain tissues at controlled temperatures, and facilitate the clearance of metabolic byproducts, thereby optimizing tissue viability during preservation. The comparatively poorer outcomes observed in major extremity replantation and transplantation, when contrasted with finger amputations, are largely attributable to the greater muscle mass involved, which predisposes these tissues to more severe IRI and a heightened systemic inflammatory response. In turn, this inflammatory cascade may lead to failure of the transplanted extremity and, in severe cases, patient mortality.[5]

This study aims to evaluate the efficacy of ECP in preserving tissue viability over time by quantitatively comparing it with CIT, with a particular focus on early reperfusion-phase changes, including inflammatory responses, oxidative stress, histopathologic alterations, and biochemical disturbances.

MATERIALS AND METHODS

A rat hindlimb ischemia model was designed to simulate IRI. To minimize surgical invasiveness and procedure-related complications, and to specifically focus on early postreperfusion changes, a model involving complete cessation and subsequent

Received December 18, 2025; and accepted for publication, after revision, February 21, 2026.

From the [a]Department of Plastic, Reconstructive and Aesthetic Surgery, Ankara Training and Research Hospital, Ankara, Turkey; [b]Department of Physiology, Hacettepe University Faculty of Medicine, Ankara, Turkey; [c]Department of Physiology, Balıkesir University Faculty of Medicine, Balıkesir, Turkey; [d]Department of Pediatrics, Hacettepe University Faculty of Medicine, Pediatric Pathology Unit, Ankara, Turkey; [e]Department of Pathology, Hacettepe University Faculty of Medicine, Ankara, Turkey; and [f]Department of Plastic, Reconstructive and Aesthetic Surgery, Hacettepe University Faculty of Medicine, Ankara, Turkey.

This study was supported by Scientific Research Committee of Hacettepe University (number 20512).

This study was approved by Animal Experimentations Local Ethics Board of Hacettepe University (Approval no: 2022/09-03).

The manuscript was presented at the 46th Annual Meeting of the Turkish Society of Plastic Reconstructive and Aesthetic Surgery in Antalya, Turkey.

Conflicts of interest and sources of funding: none declared.

Reprints: Suleyman Yildizdal, MD, Department of Plastic, Reconstructive and Aesthetic Surgery, Ankara Training and Research Hospital, Ankara, Turkey. E-mail: suleymanyildizdal@gmail.com.

Supplemental Digital Content is available for this article. Direct URL citations are provided in the HTML and PDF versions of this article on the journal's website, www.annalsplasticsurgery.com.

ISSN: 1536-3708/26/9605–0455
DOI: 10.1097/SAP.0000000000004725

restoration of blood flow to the rat hindlimb was used rather than performing isogenic transplantation or replantation.

Fourteen male Wistar Albino rats (aged 6–8 weeks; body weight 200–250 g) were randomly assigned to either the cold ischemic transport group (CIT, n = 7) or the extracorporeal perfusion group (ECP, n = 7). On the basis of the 4R principle and a review of previously conducted IRI experiments, in which the number of animals per group ranged between 5 and 8, we determined the group size as 7. Following the experiment, we observed that the effect size calculated from our significant results reached 2.04, with the observed power approaching 0.94. All experimental procedures were approved by the local institutional ethics committee (Approval no: 2022/09-03).

Surgical Procedures

The animals were anesthetized with intraperitoneal ketamine–xylazine (90/10 mg/kg). During the 6-hour ischemia period followed by 1 hour of reperfusion, maintenance doses of anesthesia were administered as needed, based on the presence of whisker movement. Before any surgical intervention, a 1 mL blood sample was obtained from the tail vein following induction of anesthesia. Core body temperature was continuously monitored using a rectal probe (YSI Tele-Thermometer, USA) and maintained with a heating pad and heating lamp. All surgical procedures were performed under 3× loupe magnification. A schematic summary of the surgical protocol is provided in Supplemental Digital Content 1, http://links.lww.com/SAP/B250.

Amputation Steps for Both Group

A 3-cm oblique skin incision was made on the right lower extremity, parallel to the inguinal ligament. The femoral artery and vein were subsequently identified and meticulously dissected (Fig. 1A). To simulate right hindlimb amputation, the isolated femoral vessels were clamped proximally, just above the level of the inguinal ligament, thereby interrupting circulation. This was followed by a transfemoral amputation that included all soft tissues and bone, while preserving the continuity of the femoral artery and vein. These initial procedural steps were identical in both experimental groups.

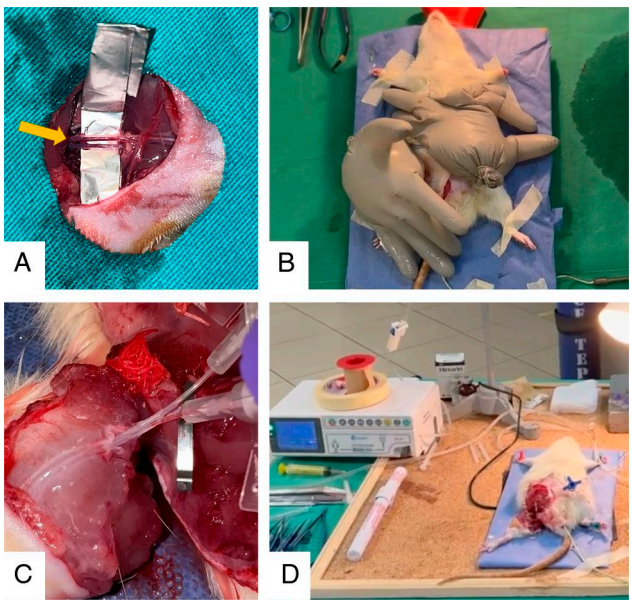

FIGURE 1. A, Femoral artery and vein were dissected. B, Amputated hindlimb placed in ice-cold water without direct physical contact. C, Artery and vein were cannulated. D, Ischemic hindlimb was perfusated using the pump.

Cold Ischemic Transport Group (CIT)

The amputated limb, with circulation occluded using a vascular microclamp, was wrapped in a waterproof sheath and submerged in ice-cold water for 6 hours, ensuring no direct contact with ice (Fig. 1B). Following the cold ischemic period, the vascular clamps were released to restore perfusion to the extremity. Reperfusion was confirmed using a milking maneuver and was maintained for 1 hour.

Extracorporeal Perfusion Group (ECP)

The femoral artery and vein were cannulated (Fig. 1C), with the femoral artery connected to the perfusion system and venous outflow established through the cannulated femoral vein. On the basis of previous reports demonstrating superior osmotic regulation and tissue preservation,[6,7] a dextran-containing Perfadex® solution was used as the perfusate during the 6-hour ischemic period, delivered via a perfusion pump (Fig. 1D) (Volumat MC Agilia, Fresenius Kabi Else-Kröner-Strabe, Hamburg, Germany). Perfusion was initiated at a flow rate of 0.1 mL/min and gradually increased to 0.3, 0.5, and 1.0 mL/min at 15-minute intervals (Fig. 2). The target flow rate of 1.0 mL/min was selected based on prior studies demonstrating maintenance of perfusion pressure and volume within physiological limits and was subsequently maintained throughout the procedure to preserve physiological conditions.[6–8] The perfusion solution was supplemented with antibiotics (1 mg sulbactam and 0.5 mg ampicillin per liter) and 5000 IU of heparin. Subnormothermic conditions were maintained at 28°C using a thermostat, as subnormothermia has been shown to reduce IRI and improve tissue outcomes.[4,9,10] Following completion of the ischemic period, vascular clamps and cannulas were removed to restore perfusion of the extremity. When necessary, minor bleeding at the cannulation sites was controlled with a single 12-0 nylon suture. Reperfusion was confirmed using a milking maneuver and was maintained for 1 hour.

After completion of the experimental protocols in both the CIT and ECP groups, 2 mL of blood was obtained via transcardiac puncture, and the animals were euthanized by anesthetic overdose. Immediately thereafter, gastrocnemius muscle samples from both the ischemia-reperfusion–treated (right) and contralateral control (left) hindlimbs were harvested, snap-frozen in liquid nitrogen, and stored at −80°C for subsequent microscopic and biochemical analyses. Skin samples obtained at the level of the gastrocnemius muscle were processed for hematoxylin and eosin (H&E) staining to assess apoptosis and inflammatory changes.

Oxidative Stress Parameters

To assess oxidative status, the total oxidant status (TOS) (*E-BC-K802-M, Elabscience*), total antioxidant status (TAS) (*E-BC-K801-M, Elabscience*), catalase (*E-BC-K031-M, Elabscience*), superoxide dismutase (SOD) (*E0168Ra, Bt-Lab*), and total thiol (*E-BC-K265-M, Elabscience*) levels were measured using the ELISA method.

Inflammatory and Biochemical Parameters

The levels of IL-10 (anti-inflammatory cytokine) (E0108Ra, Bt-Lab), TNF-α (E0764Ra, Bt-Lab), and NF-κB (inflammatory markers) (sc-8008, Santa Cruz) were evaluated with ELISA method for inflammatory changes. Biochemical parameters were

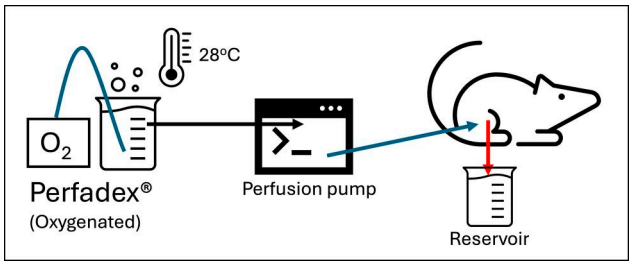

FIGURE 2. The perfusion solution was oxygenated and stored under subnormothermic conditions. Subsequently, the experimental limb was perfused via the femoral artery using a perfusion pump at a maximum flow rate of 1 mL/min. Following perfusion, the effluent was drained through the femoral vein.

measured from blood samples obtained at the beginning and end of the experiment. Biochemical parameters lactate, creatine kinase (CK), myoglobin, and lactate dehydrogenase (LDH) were measured from blood samples obtained at the beginning and end of the experiment by autoanalyzer.

Histopathologic and Apoptotic Parameters

Muscles samples were sectioned with cryostat microtome, and stained with H&E and cytochrome-c-oxidase (COX) to examine tissue damage and mitochondria, respectively. Muscle fiber damage (eosinophilic staining, loss of striation, sarcoplasmic disintegration), regenerating fibers, congestion-extravasation, interstitial edema, inflammatory cell infiltration, fibrosis, and mitochondrial changes (presence of large mitochondria, abnormal distribution within the fiber, and COX deficiency: pale/negative COX staining) were evaluated. Each parameter was scored on a scale from 0 to 3 (0: Absent, 1: Focal/mild, 2: Moderate, 3: Diffuse/severe).

Apoptosis markers bcl-2 (*E0037Ra, Bt-Lab*) and bax (*E0034Ra, Bt-Lab*) proteins were measured by ELISA in the muscle tissue. Skin samples taken from both the experimental and control contralateral limbs were stained with H&E to determine inflammation and apoptosis.

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics v23. As our data showed significant deviations from normality by the Shapiro-Wilk test and the number of subjects was low, Mann-Whitney *U* and Wilcoxon signed-rank test were used for pair-wise comparisons within and between groups.

Blood and/or muscle parameters related to oxidant status, inflammatory and apoptotic markers, biochemical measurements, and histopathologic evaluations were statistically analyzed based on within-group (pre- vs. postprotocol and control vs. experimental limb) and between-group (CIT vs. ECP) comparisons.

RESULTS

Oxidative Stress

Oxidative stress parameters TAS and TOS, representing the overall antioxidant, and oxidant systems, respectively, and SOD and total thiol levels in both plasma and muscle samples and catalase in muscle samples were presented in Table 1. In summary, the TAS values were significantly higher, and the TOS values were significantly lower in the ECP group compared with the CIT group. No significant difference was observed between the groups for antioxidant enzyme catalase. However, SOD, another antioxidant enzyme, and thiol, an antioxidant-active group, showed higher in both blood and muscle tissues in the ECP group compared with the CIT group.

Apoptosis Parameters

The values of Bax and Bcl-2 proteins measured in muscle tissue revealed similar results between groups (Table 2).

Histopathologic Parameters

Microscopic examination demonstrated more extensive, though mild, muscle fiber damage in the CIT group compared with the ECP group. None of the samples showed evidence of regenerated fibers, congestion, fibrosis, or COX deficiency.

Semiquantitative findings are summarized in Figure 3. Interstitial edema in the ECP group was significantly greater in the experimental leg than in the control leg ($P = 0.027$). In the CIT group, perivascular inflammatory cell infiltration was more pronounced in the experimental leg than control leg, approaching statistical significance ($P = 0.056$).

Cox staining revealed a higher incidence of abnormal mitochondrial distribution—particularly subsarcolemmal accumulation—in the CIT group compared with the ECP group ($P = 0.009$). In addition, the presence of large mitochondria was significantly higher in the CIT experimental leg compared with both the ECP group ($P = 0.010$) and the CIT control leg ($P = 0.018$) (Fig. 4).

H&E staining of the skin and gastrocnemius muscle showed no significant differences in inflammation or apoptosis between the control and experimental limbs in either group.

Inflammatory Changes

The levels of anti-inflammatory cytokine IL-10 and the proinflammatory cytokines TNF-α and NF-κβ measured in muscle and blood are summarized in Table 3. The results demonstrated that, significantly higher IL-10 and lower TNF-α and NF-κβ levels in the ECP group compared with the CIT group. These findings suggest a more favorable inflammatory profile in the ECP model.

Biochemical Parameters

Our results indicate an overall rise in CK, myoglobin, lactate, and LDH levels in both groups, being more prominent in the CIT than ECP group based on pre- and postischemia blood samples (Table 4).

DISCUSSION

Although CIT remains the most widely used method for tissue preservation, it is inherently associated with progressive depletion of cellular energy reserves and intracellular accumulation of metabolic byproducts.[11] Upon reperfusion, the sudden release of these accumulated metabolites into the systemic circulation precipitates IRI. Consequently, CIT alone is insufficient to adequately prevent IRI, highlighting the need for alternative preservation strategies such as ECP.

ECP systems, initially developed and widely applied in cardiovascular surgery, provide continuous delivery of oxygen and essential metabolites to preserved tissues. Modified ECP platforms have subsequently been adapted for use in solid organ transplantation, enabling prolonged tissue viability under minimal ischemic conditions and resulting in reduced cellular injury compared with CIT.[1,2,12] However, several limitations have been reported, including technical challenges during transport within the perfusion circuit, difficulties related to device connectivity,

TABLE 1. Oxidative Stress Parameters

	Blood				Muscle			
Marker	**Preoperative**	**Postoperative**	**Difference (%)**	***P***	**Experimental**	**Control**	**Difference (%)**	***P***
TOS (H_2O_2 equi/g)								
CIT	4.08 ± 0.43	8.71 ± 0.44	53.2	0.056	10.14 ± 0.27	5.86 ± 0.45	73	0.048*
Perfusion	4.33 ± 0.38	7.92 ± 0.4	45.3		8.71 ± 0.44	6.09 ± 0.45	43	
TAS (mM Trol. equi/L)								
CIT	6.15 ± 0.54	5.51 ± 1.04	−11.6	0.018*	3.4 ± 0.3	6.43 ± 0.41	−47.1	0.009*
Perfusion	5.97 ± 0.59	7.5 ± 0.56	20.4		8.57 ± 0.21	7.14 ± 0.33	20	
SOD (U/mg or U/mL)								
CIT	192 ± 30.83	179 ± 14.66	−7.3	0.028*	2.49 ± 0.29	4.11 ± 0.18	−39.4	0.002*
Perfusion	188 ± 15.64	232 ± 20.03	18.9		4.32 ± 0.22	3.92 ± 0.32	10.2	
Total Thiol (U/g or U/L)								
CIT	343 ± 30.47	201 ± 25.63	−70.6	0.002*	243 ± 35.05	262 ± 20.71	−7.4	0.006*
Perfusion	332 ± 67.88	360.5 ± 68.19	7.9		302 ± 21.25	280 ± 15.51	7.9	
Catalase (U/mg)								
CIT					156 ± 15.08	159 ± 15.4	−1.9	0.223
Perfusion					151 ± 13.25	158 ± 22.38	−4.4	

All values were given mean ± SD. Difference (%) of blood was calculated (postoperative—preoperative)/postoperative, and difference (%) of muscle was calculated (experimental—control)/control. Only mean values were used for these formulas.

and limited operator experience with perfusion systems.[13,14] In addition, the large size and complex anatomy of extremities may further complicate handling and transport. The high cost of commercially available machine perfusion devices also represents a significant barrier to widespread adoption. Nevertheless, despite these limitations, ECP systems have the potential to improve outcomes in limb replantation. Given the high metabolic demands of skeletal muscle during extremity transport, we hypothesized that ECP could attenuate IRI during limb preservation and transport.

Previous perfusion studies have investigated various experimental models using solutions with differing compositions. The primary objectives of these perfusates include preservation of composite tissue viability, regulation of electrolyte and nutrient balance, reduction of cellular death, and prevention of tissue edema. Some of these solutions contain cellular components derived from different species, including rodents, pigs, or humans. However, prior studies have demonstrated that perfusates containing cellular elements may provoke immune responses, thereby negatively affecting tissue survival. In addition, solutions containing blood-derived cellular components are associated with higher costs and logistical challenges related to storage and transport when compared with acellular solutions.[15,16] Among acellular perfusates, solutions enriched with osmotic regulatory agents have been shown to be more effective for tissue preservation and transport. For this reason, an osmotically active, acellular solution was selected in the present study. Within this category, dextran represents a key component due to its ability to preserve endothelial integrity, regulate leukocyte adhesion, maintain intravascular plasma volume, reduced muscle damage, and improved microcirculatory preservation compared with alternative perfusates.[7,8] On the basis of these findings, a dextran-containing Perfadex® solution was chosen in this study to evaluate inflammation and inflammation-related changes following ischemia-reperfusion.

The efficacy of ECP in mitigating limb ischemia has been demonstrated in previous studies, particularly in large-animal models such as pigs. Although the rat hindlimb model is cost-effective, it does not fully replicate the physiological and anatomic complexity of human extremities. Therefore, the present experimental design does not incorporate complete replantation or transplantation with vascular anastomosis, but was intentionally structured to isolate IRI while minimizing microsurgical variability and procedure-related confounders. This simplified and highly reproducible model allows focused evaluation of early ischemia-reperfusion mechanisms relevant to extremity transport. Accordingly, it primarily reflects the ischemic transport phase of extremity replantation and transplantation rather than the microsurgical reconstruction phase, and clinical extrapolation should be undertaken with caution.

Our results demonstrated a more favorable oxidative status in the groups treated with perfusion-based preservation systems. These findings are consistent with previous IRI studies investigating protective mechanisms against reperfusion-related oxidative damage.[17,18] According to these reports, interventions aimed at reducing oxidative stress significantly modulate TAS and TOS values. Notably, enzymatic markers of oxidative injury have not been extensively evaluated in limb-specific IRI models. In a study examining total thiol levels in a rat hindlimb IRI model, total thiol levels were significantly higher in the antioxidant-treated group compared with the IRI group.[19] Similarly, another study demonstrated that in experimental models where the oxidative system was "knocked out," SOD levels increased with antioxidant treatments, while catalase levels remained unchanged.[20] Catalase activity is known to be relatively high in organs such as the liver but considerably lower in the heart and skeletal muscle, which may limit the detectability of changes in muscle tissue unless more advanced stages of oxidative stress are present.[21–23] Accordingly, the absence of

TABLE 2. Apoptosis Parameters

	Muscle			
Marker	**Experimental**	**Control**	**Difference (%)**	***P***
Bcl-2 (ng/mL)				
CIT	2.86 ± 0.27	2.80 ± 0.29	2.1	0.128
Perfusion	2.71 ± 0.23	2.64 ± 0.28	2.6	
Bax (ng/mL)				
CIT	2.50 ± 0.23	2.34 ± 0.23	6.8	0.146
Perfusion	2.68 ± 0.26	2.48 ± 0.26	8	

All values were given mean ± SD. Difference (%) of muscle was calculated (experimental—control)/control. Only mean values were used for this formula.

Copyright © 2026 Wolters Kluwer Health, Inc. All rights reserved.

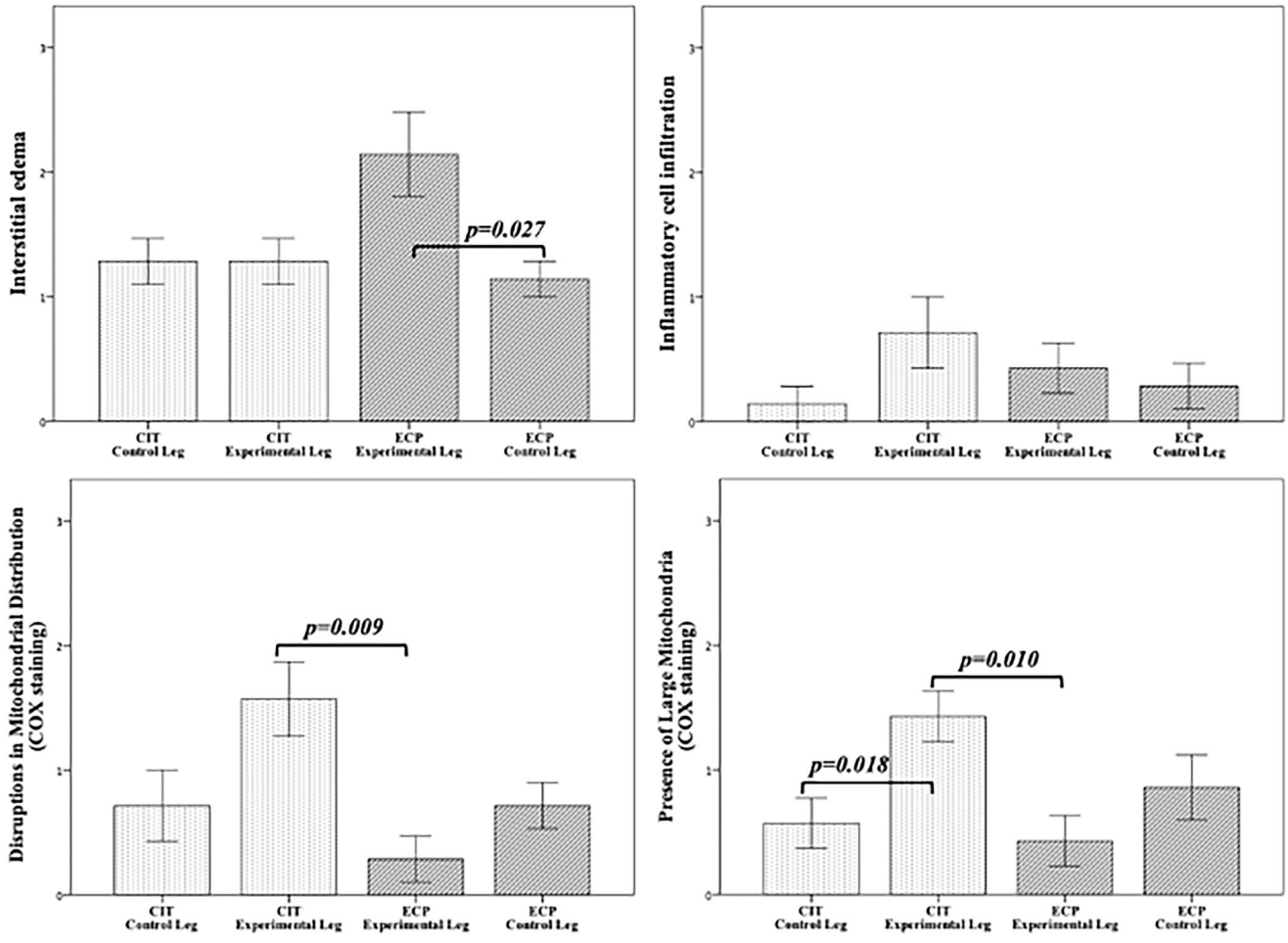

FIGURE 3. The semiquantitively scores of IR-applied and control limbs of the CIT and ECP groups in terms of interstitial edema, inflammatory cell infiltration, mitochondrial distribution, and presence of larger mitochondria.

significant changes in catalase levels observed in the present study may be attributed to the inherently low baseline catalase activity in skeletal muscle and/or the early-phase assessment of IRI, which may not have allowed sufficient time for measurable alterations to occur.

In the present study, no significant differences were observed in apoptosis marker levels between the ECP and CIT groups. This result may be attributed to the short ischemia-reperfusion times in our study. This finding is likely attributable to the relatively short ischemia and reperfusion durations used in the experimental protocol. Previous studies have demonstrated that apoptotic processes, including alterations in Bcl-2, Bax, and other apoptosis-related markers, typically become evident after 12–48 hours of reperfusion, even in tissues with high sensitivity to ischemia such as the brain.[24] A study in a porcine experimental IRI model reported that apoptosis became detectable after 24 hours of ischemia and 12 hours of reperfusion.[9] In contrast, histopathologic evaluation following 6 hours of ischemia and 1 hour of reperfusion in the present study revealed less pronounced mitochondrial swelling, hyperplasia, and redistribution in the perfused group compared with the CIT group. These observations are consistent with prior findings demonstrating reduced muscle fiber damage and fewer mitochondrial alterations in Perfadex®-perfused tissues compared with those preserved using CIT.[2] Given that mitochondrial injury represents one of the earliest cellular manifestations of IRI and can be detected within the first hour of reperfusion, these results support the interpretation that ECP primarily mitigates early-phase cellular injury. Accordingly, the reperfusion period in this study was intentionally limited to 1 hour to focus on early ischemia-reperfusion events. Early oxidative stress, inflammatory responses, and mitochondrial alterations are known to occur during this initial reperfusion phase, whereas delayed apoptotic and functional changes typically emerge at later time points and were therefore beyond the scope of the present investigation.

In the present study, levels of IL-10, a key anti-inflammatory cytokine, were significantly higher in both muscle tissue and systemic circulation in the ECP group compared with the CIT group. These findings are consistent with previous reports in IRI models. For example, IL-10 administration has been shown to markedly reduce inflammatory responses, improve histopathologic outcomes, and decrease oxidative stress markers in experimental IRI settings.[25] Furthermore, studies involving IL-10–deficient mice have demonstrated significantly exacerbated inflammatory responses and increased tissue damage following IRI compared with IL-10–competent controls.[26] In a rat hindlimb IRI model, treatment with anti-inflammatory agents was

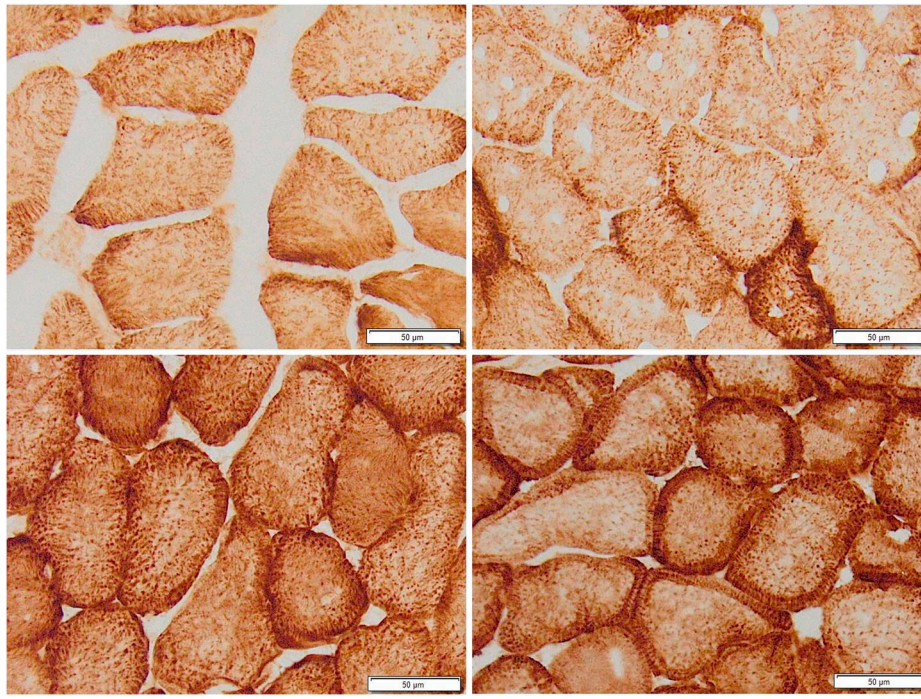

FIGURE 4. COX stain of gastrocnemius muscle from ECP (upper row) and CIT groups (lower row). (Scale bars: 50 μm).

associated with reduced expression of proinflammatory mediators, including IL-1, IL-6, TNF-α, and NF-κB, resulting in attenuation of both local and systemic inflammatory responses. This reduction was accompanied by decreased lung injury and diminished inflammatory cell infiltration within pulmonary tissue.[27] Collectively, these findings highlight the systemic protective role of elevated IL-10 levels and concomitant suppression of TNF-α and NF-κB signaling, reflecting a reduced inflammatory burden and a potentially lower risk of remote organ damage and systemic complications.

The increase in biochemical markers of tissue damage, CK, lactate, myoglobin, and LDH, was less pronounced in the ECP group. Similarly, a study on porcine limb amputation comparing perfusion and cold-storage models reported that myoglobin and potassium levels increased more in the cold-storage group than in the perfusion group. In our rat model, the relatively greater increase in biochemical parameters than porcine model may be attributable to the faster metabolic rate of rats compared with larger mammals.[2]

Inflammatory damage occurring in the early stages following extremity replantation or transplantation can lead to multiorgan failure, acute and chronic rejection, and eventually results in the loss of the transplanted tissue or even the recipient's death.[5] To reduce the risk of acute and chronic rejection and minimize the side effects of immunosuppressive drugs, preventive strategies targeting early inflammatory pathways are essential. Among these, mitigation of IRI is of particular importance, as IRI plays a central role in early postoperative tissue damage and systemic inflammatory activation. IRI remains one of the primary causes for mortality, morbidity, and extremity loss in lower extremity replantation and allotransplantations due to higher muscle load. Mortality and morbidity are caused by triggered systemic inflammatory response that leads to multiorgan failure.[5,28] In the present experimental amputation model, we demonstrated the

TABLE 3. Inflammatory Parameters

	Blood				Muscle			
Marker	**Preoperative**	**Postoperative**	**Difference (%)**	***P***	**Experimental**	**Control**	**Difference (%)**	***P***
TNF-α (ng/mg or ng/mL)								
CIT	31.00 ± 1.68	38.69 ± 3.07	19.9	$P = 0.037$*	32.20 ± 2.09	24.90 ± 3.19	29.3	$P = 0.043$*
Perfusion	31.30 ± 1.92	36.30 ± 1.67	13.7		27.80 ± 2.11	23.20 ± 1.43	19.8	
NF-κβ (pg/mg or pg/mL)								
CIT	86.00 ± 8.92	116.50 ± 14.58	26.2	$P = 0.047$*	128.00 ± 9.15	94.00 ± 8.33	36.2	$P = 0.035$*
Perfusion	88.00 ± 8.96	109.00 ± 13.22	19.3		111.00 ± 10.34	97.00 ± 6.43	14.4	
IL-10 (ng/mg or ng/mL)								
CIT	43.50 ± 4.86	50.85 ± 5.35	14.4	$P = 0.039$*	33.40 ± 3.33	38.70 ± 2.57	−13.7	$P = 0.002$*
Perfusion	43.80 ± 3.80	54.60 ± 4.57	19.8		44.40 ± 2.69	37.90 ± 3.21	17.1	

All values were given mean ± SD. Difference (%) of blood was calculated (postoperative—preoperative)/postoperative and difference (%) of muscle was calculated (experimental—control)/control. Only mean values were used for these formulas.

Copyright © 2026 Wolters Kluwer Health, Inc. All rights reserved.

TABLE 4. Biochemical Parameters

	Blood			
Marker	**Preoperative**	**Postoperative**	**Rate of Change**	***P***
CK-total				
CIT	28 ± 3	174 ± 16	6.2	0.506
Perfusion	25 ± 2	142 ± 13	5.7	
Myoglobin				
CIT	47 ± 3	342 ± 32	7.3	0.032*
Perfusion	50 ± 4	220 ± 21	4.4	
Lactate				
CIT	1.7 ± 0.2	3.8 ± 0.3	2.2	0.302
Perfusion	1.8 ± 0.2	3.4 ± 0.3	1.9	
LDH				
CIT	252 ± 24	3250 ± 238	12.9	0.048*
Perfusion	240 ± 21	2450 ± 206	10.2	

All values were given mean ± SD. Rate of change was calculated postoperative/preoperative. Only mean values were used for this formula.

superiority of ECP over CIT across multiple parameters. Specifically, ECP was associated with reduced oxidative stress and attenuated inflammatory responses, even during the early phase following reperfusion in a rat hindlimb model. Notably, findings from both experimental groups suggest that oxidative stress and inflammatory processes initiated during the early reperfusion period may have significant implications for late-term tissue viability and functional muscle integrity in replanted or transplanted extremities. On the basis of these observations, preservation of ischemic amputated tissues using ECP systems during replantation of high muscle-mass extremities or in allotransplantation procedures may help prevent long-term tissue injury, systemic inflammatory response syndrome, and remote organ damage. Ultimately, this approach has the potential to substantially improve the success rates of these complex reconstructive procedures.

This study has several limitations. First, the relatively small sample size (n = 7 per group), although consistent with previous experimental limb ischemia and perfusion studies, limits the generalizability of the findings and reflects the ethical constraints inherent to animal research. Second, the experimental model did not include full limb replantation or transplantation with vascular anastomosis; instead, it was intentionally designed to isolate IRI while minimizing microsurgical variability and procedure-related confounders. Although our surgical model demonstrates high reproducibility, the design of the study as a pure IRI model may constrain its clinical applicability. Third, the analysis was limited to a single early reperfusion time point. While this design allowed focused evaluation of acute oxidative, inflammatory, and mitochondrial changes following reperfusion, it did not permit assessment of delayed apoptotic processes, functional outcomes, or long-term tissue viability. Therefore, although the early protective effects of extracorporeal perfusion observed in this model are promising, their impact on long-term functional outcomes remains to be determined and warrants further investigation with extended reperfusion periods and functional assessments. Finally, no supplementary video, Supplemental Digital Content 1, http://links.lww.com/SAP/B250 demonstrating the extracorporeal perfusion setup and microscopic vessel cannulation was included in this study.

CONCLUSION

Clinically, the lower success rates of major extremity replantation and allotransplantations compared with finger amputations and solid organ transplantations are primarily attributed to the larger muscle mass and prolonged cold ischemia, which starts IRI and induces systemic inflammatory response. The promising results of our experimental study suggest that the clinical use of extracorporeal circulation machines for extremity transport may reduce histopathologic damage, oxidative stress, and inflammatory response.

ACKNOWLEDGMENTS

The authors report no acknowledgments.

REFERENCES

1. Ozer K. Advances in limb preservation: from replantation to transplantation. *J Hand Surg Am*. 2020;45:626–637.
2. Kueckelhaus M, Dermietzel A, Alhefzi M, et al. Acellular hypothermic extracorporeal perfusion extends allowable ischemia time in a porcine whole limb replantation model. *Plast Reconstr Surg*. 2017;139:922e–932e.
3. Akateh C, Beal EW, Whitson BA, et al. Normothermic ex-vivo liver perfusion and the clinical implications for liver transplantation. *J Clin Transl Hepatol*. 2018;6:276.
4. Burlage LC, Tessier SN, Etra JW, et al. Advances in machine perfusion, organ preservation, and cryobiology: potential impact on VCA. *Curr Opin Organ Transplant*. 2018;23:561.
5. Nasir S, Kilic YA, Karaaltin MV, et al. Lessons learned from the first quadruple extremity transplantation in the world. *Ann Plast Surg*. 2014;73:336–340.
6. Gok E, Alghanem F, Moon R, et al. Development of an ex-situ limb perfusion system for a rodent model. *ASAIO J*. 2019;65:167–172.
7. Bonen A, Clark MG, Henriksen EJ. Experimental approaches in muscle metabolism: hindlimb perfusion and isolated muscle incubations. *Am J Physiol Metab*. 1994;266:E1–E16.
8. Peoples GE, Hoy AJ, Henry R, et al. Autologous pump-perfused rat hind limb preparation for investigating muscle function and metabolism in vivo. *Microcirculation*. 2013;20:511–523.
9. Ozer K, Rojas-Pena A, Mendias CL, et al. The effect of ex situ perfusion in a swine limb vascularized composite tissue allograft on survival up to 24 hours. *J Hand Surg Am*. 2016;41:3–12.
10. RL M, HA P, Shorey W, et al. Treatment of" toxemia" after extremity replantation. *Arch Surg (Chicago, Ill 1960)*. 1964;89:871–879.
11. Gok E, Kubiak CA, Guy E, et al. Long-term effects of hypothermic ex situ perfusion on skeletal muscle metabolism, structure, and force generation after transplantation. *Transplantation*. 2019;103:2105–2112.
12. Guarrera JV, Henry SD, Samstein B, et al. Hypothermic machine preservation facilitates successful transplantation of "orphan" extended criteria donor livers. *Am J Transplant*. 2015;15:161–169.
13. Ravikumar R, Jassem W, Mergental H, et al. Liver transplantation after ex vivo normothermic machine preservation: a phase 1 (first-in-man) clinical trial. *Am J Transplant*. 2016;16:1779–1787.
14. Bral M, Gala-Lopez B, Bigam D, et al. Preliminary single-center Canadian experience of human normothermic ex vivo liver perfusion: results of a clinical trial. *Am J Transplant*. 2017;17:1071–1080.
15. Müller C, Fürst H, Reichenspurner H, et al. Lung procurement by low-potassium dextran and the effect on preservation injury. *Transplantation*. 1999;68:1139–1143.
16. Araki J, Sakai H, Takeuchi D, et al. Normothermic preservation of the rat hind limb with artificial oxygen-carrying hemoglobin vesicles. *Transplantation*. 2015;99:687–692.
17. Erer D, Dursun A, Oktar G, et al. The effects of iloprost on lung injury induced by skeletal muscle ischemia-reperfusion. *Bratisl Lek Listy*. 2014;115(7):405.–10.
18. Küçük A, Polat Y, Kılıçarslan A, , et al Irisin protects against hind limb ischemia reperfusion injury. *Drug Des Devel Ther*. 2021:361-368.https://pmc.ncbi.nlm.nih.gov/articles/PMC7871175/
19. Afolabi OA, Alabi BA, Ajike RA, et al. Evaluation of testicular torsion management in Ogbomoso, South-Western Nigeria and surgical detorsion-augmented treatment with phytochemical fractions of Corchorus olitorius leaf in experimental rats. *Saudi J Biol Sci*. 2023;30:103495.

20. Li M, Zhang Y, Zhou J, et al. Selenoprotein f knockout caused glucose metabolism disorder in young mice by disrupting redox homeostasis. *Antioxidants*. 2022;11:2105.
21. Doroshow JH, Locker GY, Myers CE. Enzymatic defenses of the mouse heart against reactive oxygen metabolites: alterations produced by doxorubicin. *J Clin Invest*. 1980;65:128–135.
22. Chen B, Tang L. Protective effects of catalase on retinal ischemia/reperfusion injury in rats. *Exp Eye Res*. 2011;93:599–606.
23. Knight KR, Messina A, Hurley JV, et al. Muscle cells become necrotic rather than apoptotic during reperfusion of ischaemic skeletal muscle. *Int J Exp Pathol*. 1999;80:169–175.
24. Ferrer I, Pozas E, Lopez E, et al. Bcl-2, Bax and Bcl-x expression following hypoxia-ischemia in the infant rat brain. *Acta Neuropathol*. 1997;94:583–589.
25. Ozturk H, Ozturk H, Terzi EH, et al. Interleukin 10 reduces testicular damage in experimental testicular ischemia/reperfusion injury. *Urology*. 2014;83:508–e1.
26. Wan X, Huang WJ, Chen W, et al. IL-10 deficiency increases renal ischemia-reperfusion injury. *Nephron Exp Nephrol*. 2014;128(1-2):37–45.
27. Xue B, Chen B, Tang Y, et al. Dexmedetomidine protects against lung injury induced by limb ischemia-reperfusion via the TLR4/MyD88/NF-κB pathway. *Kaohsiung J Med Sci*. 2019;35:672–678.
28. Yassin MMI, Harkin DW, D'Sa AABB, et al. Lower limb ischemia-reperfusion injury triggers a systemic inflammatory response and multiple organ dysfunction. *World J Surg*. 2002;26:115.

Copyright © 2026 Wolters Kluwer Health, Inc. All rights reserved.