Transrectal intracolon cooling prevents paraplegia and mortality in a rat model of aortic occlusion-induced spinal cord ischemia

Robert S. Crawford, MD,[a] Yang Liu, MD,[a] Dong Yuan, MD,[a] Chunli Liu, MD,[b] Rajabrata Sarkar, MD, PhD,[a] *and* Bingren Hu, MD, PhD,[a,b] *Baltimore, Md*

ABSTRACT

Objective: Spinal cord ischemia–reperfusion injury (SC-IRI) occurs in many medical conditions such as aneurysm surgical repair but no treatment of SC-IRI is available in clinical practice. The objective of the present study was to develop a novel medical device for the treatment of SC-IRI.

Methods: A rat model of SC-IRI was used. A novel transrectal intracolon (TRIC) temperature management device was developed to maintain an intracolon wall temperature at either 37°C (TRIC37°C) or 12°C (TRIC12°C). The upper body temperature was maintained as close as possible to 37°C in both groups. A 2F Fogarty balloon catheter was inserted via the left common carotid artery to block the distal aortic blood flow to the spinal cord. The proximal blood pressure was controlled by the withdrawal and infusion of blood via the jugular vein catheter, such that the distal tail artery blood pressure was maintained at ~10 mmHg for 13 and 20 minutes, respectively. Next, the balloon was deflated, and TRIC temperature management was continued for an additional 30 minutes to maintain the colon wall temperature at either 37°C or 12°C during the reperfusion period.

Results: All the rats subjected to 13 minutes of spinal cord ischemia in the TRIC37°C group had developed paraplegia during the postischemic phase. In striking contrast, TRIC at 12°C completely prevented the paraplegia, dramatically improved the arterial blood gas parameters, and avoided the histopathologic injuries to the spinal cord in rats subjected to 13 minutes of spinal cord ischemia. Furthermore, TRIC12°C allowed for the extension of the ischemia duration from 13 minutes to 20 minutes, with significantly reduced functional deficits.

Conclusions: Directly cooling the intestine focally with the TRIC device offered an exceptional survival rate and functional improvement after aortic occlusion-induced spinal cord ischemia. (JVS–Vascular Science 2021;2:181-93.)

Clinical Relevance: The present study showed that the use of the transrectal intracolon (TRIC) device to directly cool the intestine offers outstanding clinical benefits against spinal cord ischemia–reperfusion injury and prevents mortality in the rat aortic occlusion spinal cord ischemia model. The translational value of the present study could be high because the protection was so dramatic, the TRIC device is easy to use, and the TRIC cooling adverse effects were minimal. This novel TRIC management modality can offer fast cooling of the gut from 37°C to 12°C within 5 minutes, and the upper body temperature can be maintained in a tolerable temperature range, minimizing the fatal adverse effects of whole-body deep cooling.

Keywords: Aortic surgery; Intestinal inflammation; Ischemia–reperfusion injury; Paraplegia; Spinal cord; Therapeutic hypothermia; Transrectal intracolon device; TRIC

Spinal cord ischemia–reperfusion injury (SC-IRI) is a devastating complication of many medical conditions such as acute aortic pathology or aortic surgery.[1-6] At present, no effective treatment is available for clinical patients with SC-IRI.

Living tissue, in particular, nervous tissue, is highly susceptible to IRI under normothermic conditions.[7] Therapeutic hypothermia (TH) is the most effective treatment currently known for protecting tissues from IRI.[7] Systemic or whole-body TH can generally be divided into mild (32°-34°C), moderate (28°-31.9°C), deep (11°-28°C), profound (6°-10°C), and ultra-profound (≤5°C) according to the core temperature.[7-11] The clinical benefit of TH was often inversely related to the degree of the target temperature.[7-11] In large animal models of traumatic cardiac arrest, systemically reducing the core

From the Departments of Anesthesiology and Surgery, University of Maryland School of Medicine[a]; and the Veterans Affairs Maryland Health Center System.[b]

The present study was supported by the National Institutes of Health (grants R01 NS102815, and R01 NS097875 to B.H.), the Department of Veterans Affairs (Veterans Affairs merit award BX003926 to B.H.), and the Department of Defense (grant W81XWH1810306 to B.H.)

Author conflict of interest: none.

Correspondence: Bingren Hu, MD, PhD, Departments of Anesthesiology and Surgery, University of Maryland School of Medicine, Baltimore, MD 21201 (e-mail: bhu@som.umaryland.edu).

The editors and reviewers of this article have no relevant financial relationships to disclose per the JVS-Vascular Science policy that requires reviewers to decline review of any manuscript for which they may have a conflict of interest.

2666-3503

https://doi.org/10.1016/j.jvssci.2021.07.003

temperatures to 10°C to 15°C optimally preserved tissue and promoted survival.[6-9] However, such low core temperatures (10°C-15°C) cannot be implemented in most human medical conditions because whole-body deep TH, even at 28°C, in humans is associated with fatal myocardial and pulmonary dysfunction.[9,10] Deep TH in humans can only be used with the aid of cardiopulmonary bypass (eg, during surgical thoracoabdominal aortic aneurysm repair).[10]

Given the fatal limitations of whole-body deep (11°C-28°C) TH and no therapy currently available for SC-IRI, we developed a novel transrectal intracolon (TRIC) temperature management device to manage the temperature of the intestine and its surrounding organs and tissues. The objective of the present study was to use the novel TRIC device for the management of SC-IRI. The results from the present study showed that directly cooling the gut with the TRIC device can astonishingly alleviate SC-IRI, dramatically improve functional recovery, and virtually abolish mortality in a rat model of spinal cord ischemia.

ARTICLE HIGHLIGHTS

- **Type of Research:** In the present study, we used a novel transrectal intracolon (TRIC) temperature management device for the treatment of aortic occlusion-induced spinal cord ischemia–reperfusion injury in a rat model.
- **Key Findings:** All the rats subjected to 13 minutes of spinal cord ischemia in the TRIC37°C group had paraplegia. In striking contrast, the TRIC at 12°C completely prevented paraplegia, dramatically improved the arterial blood gas parameters, and avoided the histopathologic injuries to the spinal cord in the rats subjected to 13 minutes of spinal cord ischemia. Furthermore, TRIC12°C allowed for extension of the ischemia duration from 13 minutes to 20 minutes, with a significantly reduced incidence of functional deficits.
- **Take Home Message:** Directly cooling the intestine focally with the TRIC device resulted in an exceptional survival rate and functional improvement after aortic occlusion-induced spinal cord ischemia.

METHODS

Ethics statement. The Animal Use and Care Committee at the University of Maryland School of Medicine reviewed and approved the animal protocol, which conforms to the Guide for the Care and Use of Laboratory Animals by the U.S. National Institutes of Health (Bethesda, Md).

Rat spinal cord ischemia model. The flowchart showing the study protocol is presented in Fig 1.

Rats. Male Sprague-Dawley rats weighing ~450 g were purchased from Charles River (Boston, Mass). The rats were kept under standardized conditions of temperature (22°C ± 1°C), humidity (55% ± 5%), and 12:12-hour light and dark cycles with ad libitum access to food and water. Bedding material was placed in each cage. The animal care and laboratory staff conducted routine husbandry procedures (eg, cage cleaning, feeding, replenishing water) and checked the rats daily to assess their conditions.

Anesthesia. The rats were anesthetized throughout the surgery with 1.5% to 2% isoflurane in a mixture of nitrous oxide/oxygen (70:30) delivered via an endotracheal tube and connected to a rat ventilator (tidal volume, 2.5-3.5 mL; respiration rate, 65 breaths/minute; Ugo Basile, Varesa, Italy).

Aseptic surgical procedure. Small incisions in the neck, axillary, and tail areas were made under aseptic conditions under sterile surgical draping. The instruments used included (1) a 2F Fogarty balloon catheter (Edwards Life Sciences, Irvine, Calif) inserted via the common carotid artery; (2) a jugular vein catheter (catalog no. 508-003; Silastic; Dow Corning Corp, Midland, Mich); (3) a left axillary arterial catheter (PE-10); and (4) a tail arterial catheter (PE-50). Heparin (30 U/kg) was administered intra-arterially 5 minutes before aortic occlusion.

Spinal cord ischemia. The rat spinal cord ischemia model was produced by aortic occlusion combined with systemic hypotension. Systemic hypotension was induced by blood withdrawn via the jugular vein catheter for 2 to 4 minutes, which was immediately followed by inflation of the aortic balloon. The 2F Fogarty balloon catheter was inserted via the common carotid artery and further advanced into the descending thoracic aorta according to a previously described method.[11,12] The catheter balloon was first partially inflated to occlude both the aortic arch and the subclavian artery. Occlusion of the subclavian artery was confirmed by the reduction in the mean arterial blood pressure (MAP), measured from the left axillary artery catheter. The tail artery MAP was reduced to <50 mm Hg by withdrawing blood via the jugular vein catheter, which required ~3 minutes to accomplish and was immediately followed by full inflation of the balloon with 1.0 mL of water. The withdrawn blood volume was 7.24 ± 1.47 mL for the TRIC37°C group and 7.09 ± 1.08 mL for the TRIC12°C group (mean ± standard error of the mean; n = 8/group; $P = .81$; two-sided t test). The distal (tail artery) MAP was maintained at 10 ± 1.0 mm Hg by withdrawing and infusing blood via the jugular vein catheter. In this condition, the proximal (right axillary artery) MAP was ~50 to 60 mm Hg. The balloon inflation and distal MAP were maintained for either 13 or 20 minutes according to the experimental group. At the end of spinal cord ischemia, the withdrawn

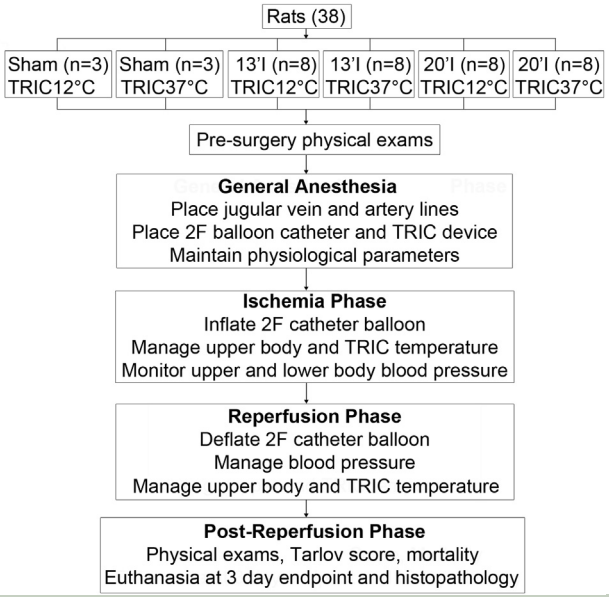


Fig 1. Flowchart showing the protocol of the study. *13'I,* 13 Minutes of spinal cord ischemia; 20'I, 20 minutes of spinal cord ischemia; *exams,* examinations; *Sham,* sham-operated rats; *TRIC,* transrectal intracolon (temperature management device).

blood was returned, and the balloon was deflated to start reperfusion.

TRIC temperature management. A TRIC device (Supplementary Fig 1) was inserted via the rectum and advanced ~10 cm in length from the anus into the descending colon to manage the temperature of the colon and the nearby thoracolumbar spinal cord to mitigate SC-IRI. A temperature probe was inserted into the descending colon to measure the intracolon wall temperature. Another temperature probe was embedded into the head temporalis muscle to measure the upper body temperature and a third probe was placed in the deep layer of the lower back (between lumbar spines 2 and 3) paraspinal muscle (~2 cm deep) to measure the spine temperature. The intracolon, upper body, and spine temperatures and the proximal and distal MAPs were recorded throughout the experiment period using the Powerlab 16-channel data acquisition system (ADInstruments, Dunedin, New Zealand). The arterial blood gas (ABG) parameters were measured via an ABL90 FLEX blood gas analyzer (Radiometer, Copenhagen, Denmark).

Experimental groups. The rats were randomly assigned identification numbers before the surgical procedures. Based on the preliminary studies, we performed a sample size estimate using the power of 0.80, indicating an ≥80% chance of detecting a difference among groups when three rats in the sham groups and five rats in the spinal cord ischemia groups were used. One rat was excluded from the study because of a surgical procedure accident (causing bleeding) and was replaced with another rat. The exclusion criteria were predefined and not determined on a post hoc basis.

Four experimental groups and two control groups were included in our spinal cord ischemia study: (1) sham surgery plus TRIC at 12°C (n = 3); (2) sham surgery plus TRIC at 37°C (n = 3); (3) 13 minutes of ischemia plus TRIC at 12°C (n = 8); (4) 13 minutes of ischemia plus TRIC at 37°C (n = 8); (5) 20 minutes of ischemia plus TRIC at 12°C (n = 8); and (6) 20 minutes of ischemia plus TRIC at 37°C (n = 8). The TRIC device was continuously activated during the period from 5 minutes before the induction of ischemia to 30 minutes of reperfusion after spinal cord ischemia to maintain the intracolon temperature at 12°C or 37°C. The upper body temperature was maintained as close as possible to 37°C in all the groups.

At the end of the cooling period, rewarming was initiated using the same TRIC device. During rewarming, the TRIC device was circulated with slightly and gradually increasing warmer water from the reservoir to control the spine temperature rewarming rate at 0.15°C/minute. When the spine temperature had reached 30°C (which required an additional 60-90 minutes), the TRIC device was stopped and removed, anesthesia was discontinued, and the rats were returned to their home cage. In the TRIC37°C groups, the TRIC device was activated in the same manner but circulated with 36°C to 37°C of water.

Survival rate, Tarlov score, and sensory function. All examinations were performed in a double-blind fashion in which the animal groups were labeled only with the animal identification numbers without the experimental conditions. The rats were monitored continuously by us from 9 AM to 6 PM during the day and using the hardware and software from the PhenoTyper infrared video recording device (Noldus, Leesburg, Va) during the night. The moribund state was defined by the following criteria: (1) the rat had not responded to stimuli for >2 hours; (2) the rat rectal temperature was <30°C; and (3) the rat oxygen saturation level was <80%. These measures were used to avoid potential postmortem histologic deterioration or damage. The times of animal death were documented. The hind limb locomotor function of the living rats was evaluated using the Tarlov score at 4, 24, 48, and 72 hours after ischemia. The following scores were used: 6, death from ischemia; 5, complete paraplegia; 4, minimal movement of the hind limb; 3, standing with assistance; 2, standing alone but unable to walk; 1, weak walking; and 0, full recovery with normal walking. The sensory function was evaluated by the reaction to the hind paw pinch: 0, normal response; 1, minor response; and 2, no response or death.

Histologic evaluation and scoring. At the endpoint, the rats were fixed in perfusion with formalin. Tissue samples

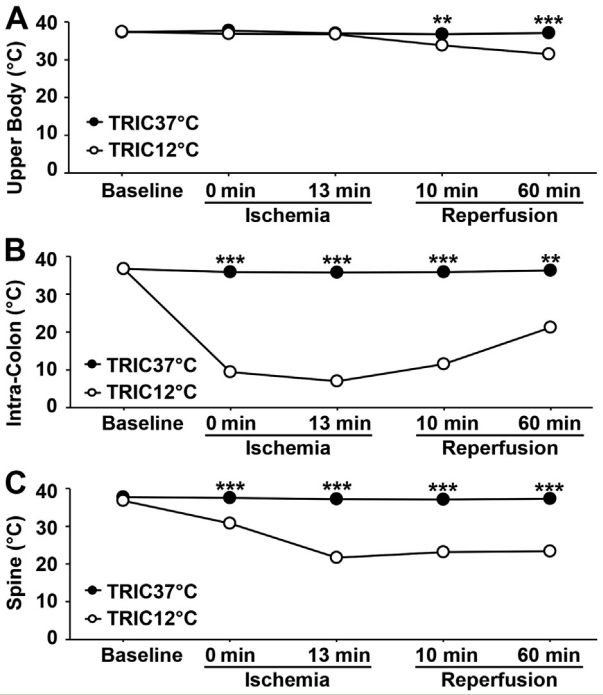


Fig 2. The upper body **(A)**, intracolon **(B)**, and spine **(C)** temperatures were recorded throughout the experiments. The rats were subjected to 13 minutes of ischemia, followed by reperfusion in the transrectal intracolon (TRIC) device at 37°C (TRIC37°C) group (*filled circles*; n = 8) and TRIC12°C group (*open circles*; n = 8), respectively. Data presented as mean ± standard error of the mean. $^{**}P <$ .005 and $^{***}P <$ .001 denotes statistically significant differences between the TRIC12°C and TRIC37°C groups; two-tailed Student's *t* test.

of the spinal cord (L5 lumbar segment), liver, intestines (duodenum and colon), and kidney were collected and embedded into paraffin. The rats in a moribund state or dead for <3 to 4 hours before the endpoint were also fixed with formalin, and the same areas of postmortem organ samples were embedded in paraffin. The formalin-fixed and paraffin-embedded tissue sections (10-μm thick) from the prescribed areas were stained with hematoxylin and eosin and examined with light microscopy. We examined more than three histological sections per rat by screening all the cross-sections with a 40× microscopic objective. A total of four to eight rats per group underwent histologic analysis among the spinal cord ischemia groups. The rats that had been dead for >4 hours were excluded from the histologic analysis to avoid potential postmortem histologic deterioration or damage. Histopathologic changes were scored by two independent investigators in a double-blind fashion. The histopathologic scoring criteria were as follows: 0, normal tissue; 1, selective cell injury in which the injured cells were scattered among the normal cells without forming an injury area (more than five cells injured in the same area); 2, forming a small (5-10 injured cells) injury area; 3, more than one small injury area or a large (>10 injured cells) injury area without tissue destruction; and 4, tissue destruction. If tissue hemorrhage was present, one point was added to the histologic score.

Statistical analysis. The data are presented as the mean ± standard error of mean. The Student *t* test (one-sided) was used for comparison of two experimental groups, and one-way analysis of variance followed by the Tukey post hoc test was used for the comparison of more than two experimental groups for statistical analysis of the blood chemical, blood gas, and animal behavioral data. The Mann-Whitney *U* test was used to analyze the Tarlov and histopathologic scores. The log-rank test was used for the survival rate analysis. Finally, the χ^2 test was used to analyze the mortality rate. *P* values <.05 were considered statistically significant.

RESULTS

TRIC temperature management. The upper body, intracolon, and spine temperatures before, during, and after induction of 13 minutes of spinal cord ischemia are shown in Fig 2. In the TRIC37°C group, circulation of the TRIC device with 36°C to 37°C water led to intracolon, upper body, and spine temperatures at a constant 36°C to 37°C range (Fig 2). In the TRIC12°C group, circulation of the TRIC device with ice-cold water resulted in a quick reduction of the intracolon temperature from 36.4°C ± 0.5°C to 10°C to 12°C within 5 minutes and a spine temperature to 21.8°C ± 1.6°C at the end of 13 minutes of ischemia (Fig 2). During the ischemic period, the upper body temperature was maintained at a 36°C to 37°C range, likely because most of the blood volume circulation to the lower body was blocked by the aortic balloon occlusion, preventing effective heat exchange via the systemic circulation between the upper and lower body. This was likely the basis for maintaining the lower temperature in the abdominal cavity and, hence, near the spinal cord. At 60 minutes of reperfusion, the upper body temperature had significantly declined from 36°C to 37°C to ~31°C and the intracolon temperature had increased from just <10°C to the 15°C to 20°C range and the spine temperature had increased from 20°C to 21°C to 23°C to 25°C (Fig 2). This followed the deflation of the aortic balloon, where reperfusion led to the warmer blood of the upper body flowing to the colder abdominal organs and, reciprocally, the colder abdominal organ blood circulating back to the upper body. This heat exchange resulted in the rebalance of the upper and lower body temperatures (Fig 2).

Effects of TRIC temperature management on MAP. The results of the quantitative analysis of the proximal and distal MAPs in the TRIC12°C and TRIC37°C groups before, during, and after spinal cord ischemia are shown in Fig 3. When blood was withdrawn from the jugular

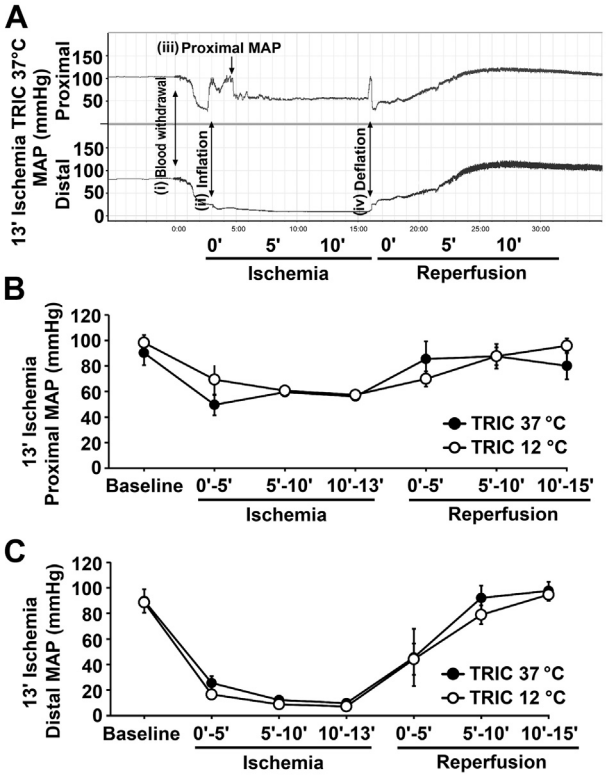


Fig 3. A, Recordings of the proximal and distal mean arterial pressures (MAPs) in the spinal ischemia model. *Arrows* denote (i) blood withdrawal to induce hypotension; (ii) inflation of the intra-aortic balloon to induce spinal cord ischemia; (iii) adjusting the proximal MAP to control the distal MAP to just <10 mm Hg to induce a consistent degree of spinal cord ischemia; and (iv) deflation of the intra-aortic balloon to start reperfusion. **B,** Proximal MAP. **C,** Distal MAP. The rats were subjected to 13 minutes of spinal cord ischemia, followed by reperfusion. Data presented as mean ± standard error of the mean. No statistically significant differences were found in the proximal and distal MAPs between the transrectal intracolon (TRIC) device at 12°C and 37°C (TRIC12°C and TRIC37°C, respectively) groups. One-way analysis of variance, followed by the Tukey test, was used for statistical analysis.

vein catheter to induce hypotension, the proximal and distal MAPs were both reduced to ~30 to 40 mm Hg within ~3 minutes (Fig 3, *A*). Inflation of the aortic balloon resulted in the proximal MAP increasing to 70 to 100 mm Hg, and the distal MAP decreasing to 15 to 20 mm Hg (Fig 3, *A*). To produce consistent spinal cord ischemia or paraplegia with rats remaining alive, the distal blood pressure was kept at just <10 mm Hg, which was accomplished by maintaining the proximal MAP at 50 to 60 mm Hg by infusion and withdrawal of blood from the jugular vein (Fig 3, *A*). At the end of 13 or 20 minutes of spinal cord ischemia, the aortic balloon was deflated (Fig 3, *A*) and the withdrawn blood was returned. Therefore, the proximal and distal MAPs both recovered to their normal baseline levels within ~10 minutes (Fig 3, *A*). The average MAP data from eight rats in each spinal cord ischemia group are shown in Fig 3, *B* and *C*. In the sham-operated groups, the proximal and distal MAPs were both slightly lower in the TRIC12°C than in TRIC37°C rats; however, the difference was not statistically significant (Supplementary Fig 2).

Effects of TRIC temperature management on ABG parameters. The ABG parameters of pH, glucose, potassium, and lactate levels in the TRIC12°C and TRIC37°C groups after 13 minutes of spinal cord ischemia are shown in Fig 4. During the initial 0- to 15-minute period of reperfusion, the pH and glucose levels tended to fall, and the potassium and lactate tended to rise, relative to the preischemic baseline levels (Fig 4). During the 15 to 60 minutes of reperfusion, the pH, glucose, and lactate levels had recovered slightly toward the preischemic baseline levels in both groups (Fig 4). The pH, glucose, and potassium levels had gradually recovered toward the baseline in the TRIC12°C group (Fig 4), but these parameters had become significantly worse in the TRIC37°C group (Fig 4). By 90 to 120 minutes of reperfusion, the pH, potassium, and lactate levels had further recovered toward the baseline levels in the TRIC12°C group. However, the recovery of these parameters was significantly slower in the TRIC37°C group (Fig 4). The greatest differences were in the glucose and potassium parameters at 120 minutes of reperfusion between the TRIC12°C and TRIC37°C groups (Fig 4, *B* and *C*).

Effects of TRIC temperature management on organ injury. Micrographs of hematoxylin and eosin–stained tissue sections of the duodenum, colon, kidneys, and spinal cord are shown in Fig 5. The quantitative analyses showed statistically significant damage to the duodenum and spinal cord; however, the differences in the damage to the colon and kidneys was not statistically significant after 13 minutes of spinal cord ischemia (Fig 5). TRIC at 12°C prevented tissue damage (Fig 5). Specifically, the duodenum epithelium damage was severe in the TRIC37°C rats (Fig 5, *A*). In contrast, no damage was seen in the TRIC12°C group after 13 minutes of ischemia (Fig 5, *A*). The colon had only minor histopathologic damage in the TRIC37°C group, with no damage seen in the TRIC12°C group (Fig 5, *B*). The intraglomerular space of the kidney appeared densely packed, and kidney tissue hemorrhage was observed in one of nine TRIC37°C rats (Fig 5, *C*). However, no kidney damage was seen in the TRIC12°C rats (Fig 5, *C*). The spinal cord damage was the most severe of the organs examined and displayed selective neuronal death (Fig 5, *D*). No small infarct foci were in the spinal cord, suggesting that no no-flow phenomenon had occurred after 20 minutes of spinal

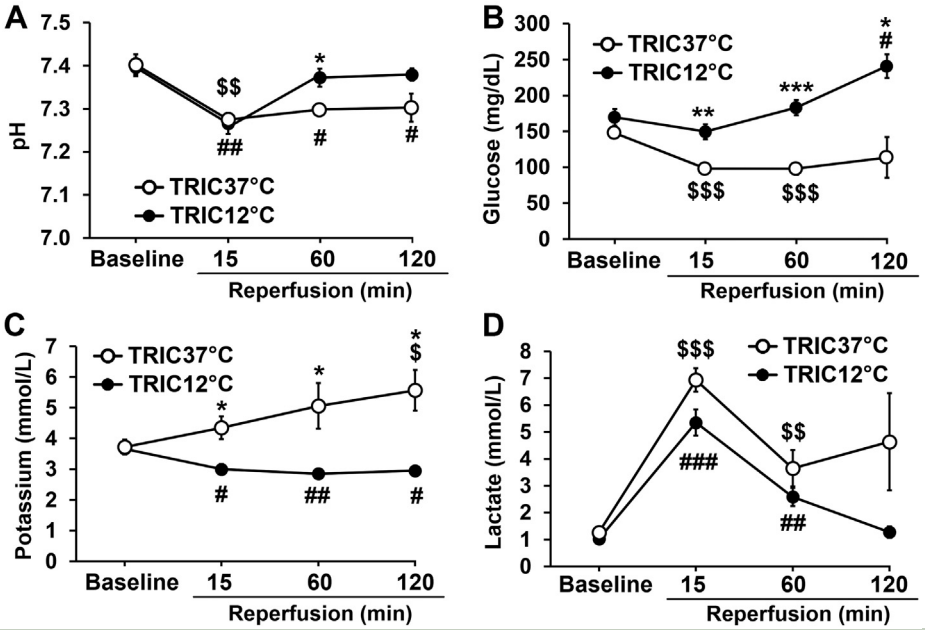


Fig 4. Arterial blood gas (ABG) parameters: pH **(A)**, glucose **(B)**, potassium **(C)**, and lactate **(D)**. Data presented as mean ± standard error of the mean. $^{\#\#}P < .01$ and $^{\#\#\#}P < .001$ for the transrectal intracolon (TRIC) device at 12°C (TRIC12°C) group (n = 8) and $^{\$\$}P < .01$ and $^{\$\$\$}P < .001$ for the TRIC37°C group (n = 8), indicating statistical significance between the baseline level and levels during ischemia or reperfusion time points. One-way analysis of variance, followed by the Tukey post hoc test, was used for statistical analysis. $^{*}P < .05$, $^{**}P < .01$, and $^{***}P < .001$ denote statistical significance between TRIC12°C and TRIC37°C groups at the same time point; the two-tailed Student *t* test was used for statistical analysis.

cord ischemia in this model. Ischemic dead neurons showed eosinophilic cytoplasm and polygonal-shaped and shrunken nuclei (Fig 5, *D*) in all TRIC37°C rats. In contrast, the spinal cord neurons were virtually normal in the TRIC12°C rats (Fig 5, *D*). In the sham-operated groups, all organ tissues and cells examined in the TRIC12°C and TRIC37°C rats were normal under light microscopy (data not shown).

Effect of TRIC temperature management on functional deficits. The motor and sensory scores were evaluated after 13 minutes of spinal cord ischemia. The TRIC37°C rats were almost 100% paraplegic (motor score, 5.6 ± 0.3) and had almost no sensory response to stimuli (sensory score, 1.9 ± 0.1; Fig 6, *A* and *B*). In contrast, both motor and sensory functions were minimally affected in the TRIC12°C rats after 13 minutes of spinal cord ischemia (Fig 6, *A* and *B*). When the spinal cord ischemia duration was extended to 20 minutes, the TRIC37°C rats developed complete paraplegia (Fig 6, *C*) and the TRIC12°C rats were partially paraplegic (Fig 6, *C*). Similarly, the sensory function was completely lost during the survival period in the TRIC37°C rats (Fig 6, *D*), and the sensory functions were partially lost in the TRIC12°C rats after 20 minutes of spinal cord ischemia (Fig 6, *D*). In the sham-operated groups, neither the motor nor sensory functions were significantly altered in the TRIC12°C and TRIC37°C groups (data not shown).

Effects of TRIC temperature management on survival rate and mortality. Approximately 56% of the rats in the TRIC37°C group survived for 24 hours after ischemia (Fig 7, *A*). In contrast, all eight rats in the TRIC12°C group survived until the endpoint of the study (Fig 7, *A*). In the 20-minute ischemia groups, none of the TRIC37°C rats survived for 4 hours, although ~45% of the rats survived for 24 hours in the TRIC12°C group.

The mortality rate was ~44% in the TRIC37°C rats vs 0% in the TRIC12°C rats after 13 minutes of spinal cord ischemia (Fig 7, *C*). In contrast, the mortality rate was 100% in the TRIC37°C rats and ~65% in TRIC12°C rats (Fig 7, *C*) after 20 minutes of spinal cord ischemia. In the sham-operated groups, all the rats in the TRIC12°C and TRIC37°C groups survived until the endpoint of the study (data not shown).

DISCUSSION

The results from the present study have shown that the use of TRIC to directly cool the intestine offers outstanding clinical benefits against SC-IRI and prevents mortality in the rat aortic occlusion spinal cord ischemia model. The translational value of the present study might be high because the protection was so dramatic, the TRIC device was relatively easy to use, and the TRIC cooling adverse effects were minimal. Even modest improvement in the resistance to spinal cord ischemia can

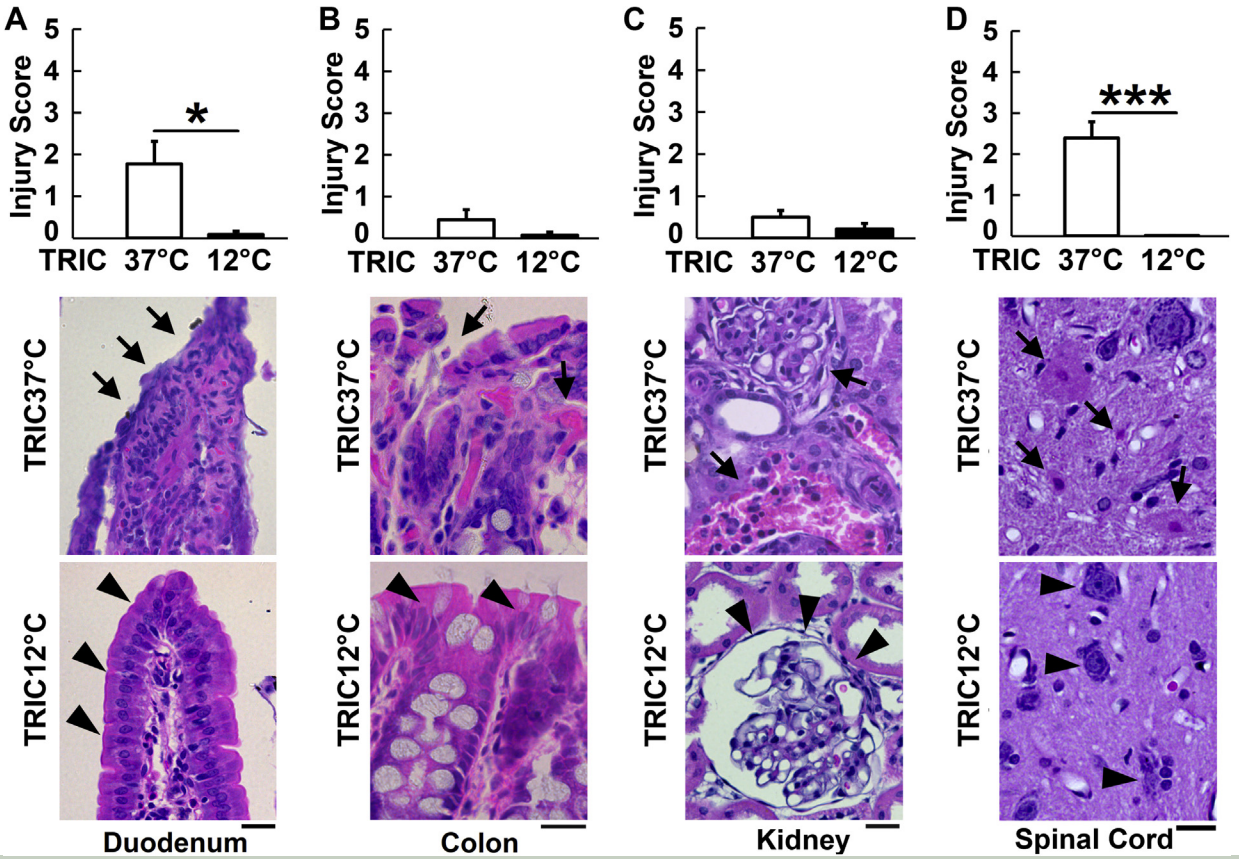


Fig 5. Hematoxylin and eosin–stained micrographs of the duodenum **(A)**, colon **(B)**, kidney **(C)**, and spinal cord **(D)** in the transrectal intracolon (TRIC) device at 37°C and 12°C (TRIC37°C and TRIC12°C, respectively) rats. The rats were subjected to 13 minutes of ischemia, followed by 3 days of reperfusion. *Arrows* indicate damaged tissue, and *arrowheads* point to normal tissue. All scale bars = 20 μm. The quantitative histologic injury scores are shown in bar graphs. Data presented as mean ± standard error of the mean. The Mann-Whitney *U* test was used to determine statistical significance between the TRIC37°C (n = 8) and TRIC12°C (n = 8) groups (*$P < .05$ and ***$P < .001$).

benefit patients with various aortic occlusion conditions such as during aneurysm surgical repair. This novel TRIC management modality can offer fast cooling of the gut from 37°C to 12°C within 5 minutes, with the upper body temperature maintained within a tolerable temperature range, minimizing the fatal adverse effects of whole-body deep cooling.

TRIC temperature management is an improved TH modality. TH remains the most promising treatment for patients with acute tissue ischemia.[6-11] The benefit of TH was often inversely related to the degree of the target temperature.[7-11] Deep TH in humans can be used with the aid of cardiopulmonary bypass for surgical thoracoabdominal aortic aneurysm repair.[10] However, without the aid of cardiopulmonary bypass, whole-body deep TH (11°C-28°C) might not be feasible for many medical conditions because it has been closely associated with fatal complications of cardiac arrest, pulmonary dysfunction, and worsened coagulopathy.[13-18] These complications were not seen during the TRIC12°C focal deep cooling period or thereafter in the present study. The reason for the lack of complications was likely because most of the body blood volume was circulating in the "warmer" upper body and the demand for blood circulation to the TRIC deep or profound cooling tissue was significantly reduced.[7-10] This is consistent with the fact that TRIC12°C significantly reverses the ischemic changes in the levels of lactate, potassium, and glucose relative to TRIC37°C (Fig 4). No sign of bleeding in the tissue or surgical incisions is consistent with the data of the prothrombin time/international normalized ratio in sham-operated rats. These findings showed only a slightly, but insignificantly, longer prothrombin time/international normalized ratio in the TRIC12°C group than in the TRIC37°C group (Supplementary Fig 3). In addition to minimizing the deep or profound cooling complications, the use of TRIC to directly cool the gut resulted in extraordinary improvement in the survival rate, tissue preservation, and motor and sensory functional recovery after aortic occlusion-induced spinal cord ischemia.

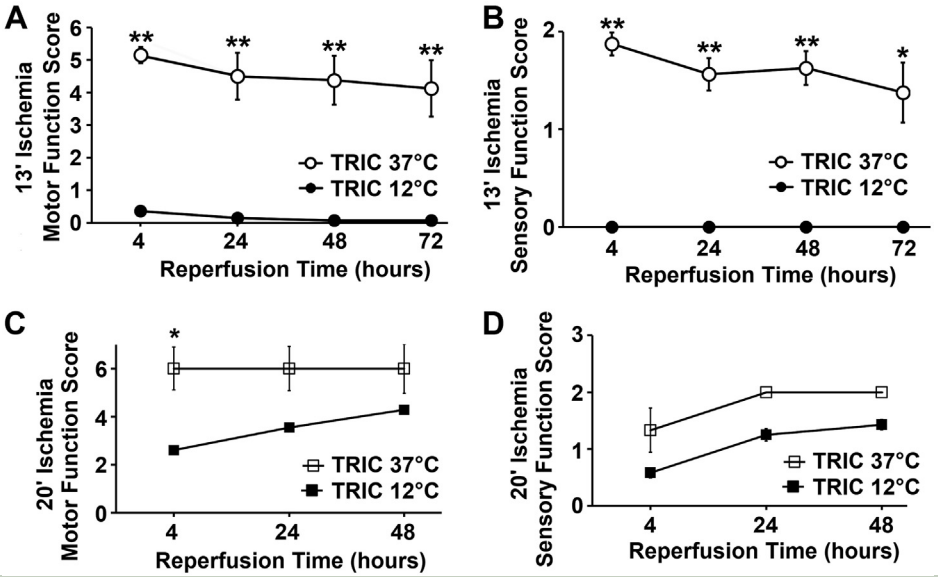


Fig 6. Motor (**A** and **C**) and sensory (**B** and **D**) function. The rats were subjected to 13 minutes of ischemia, followed by 3 days of reperfusion in the transrectal intracolon (TRIC) device at 12°C (TRIC12°C) group (n = 8) and TRIC37°C group (n = 8) or 20 minutes of ischemia, followed by 2 days of reperfusion in the TRIC12°C (n = 8) and TRIC37°C (n = 8) groups. Data presented as mean ± standard error of the mean. The Mann-Whitney *U* test was used to determine statistical significance between the TRIC37°C and TRIC12°C groups (**P* < .05 and ***P* < .01).

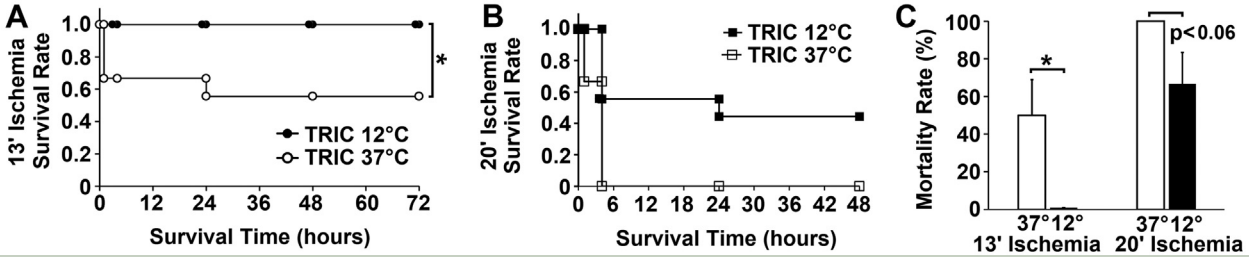


Fig 7. Survival rate and mortality. **A,** Survival rate in the transrectal intracolon (TRIC) device at 12°C (TRIC12°C) group (*filled circles*; n = 8) and TRIC37°C group (*open circles*; n = 8) after 13 minutes of ischemia. **B,** Survival rate in the TRIC12°C group (*filled squares*; n = 8) and TRIC37°C group (*open squares*; n = 8) after 20 minutes of ischemia. **C,** Mortality in TRIC37°C and TRIC12°C groups after 13 or 20 minutes of ischemia, respectively. Data presented as mean ± standard error of the mean. The log-rank test was used to determine statistical significance in the survival rate between the TRIC12°C and TRIC37°C groups (**P* < .05). The χ^2 test was used to determine statistical significance in mortality between the TRIC12°C and TRIC37°C groups (**P* < .05).

Another advantage of the TRIC device is the cooling speed, which is essential for TH, because time is the most critical factor in the treatment of tissue ischemia. Generally, the earlier the implementation of TH, the better the results for tissue protection.[7-10] At present, skin cooling and endovascular cooling are the two main TH methods.[7,9,10,18] Skin cooling is highly ineffective because of the poor heat conductance of the skin and because the subcutaneous fat shields heat transfer.[7] Endovascular cooling is invasive and also less effective, with an estimated median cooling rate of 2°C to 3°C per hour in humans.[18] In contrast, TRIC cooling can cool the gut wall from 37°C to ~10°C within 5 minutes and the spinal cord to ~22°C within 20 minutes, with the upper body maintained within a normal or tolerable temperature range.

TRIC temperature management reduces mortality. The major finding of the present study is that the TRIC fast cooling of the gut offers robust protection against SC-IRI and prevents mortality after 13 minutes of spinal cord ischemia. This could be a significant accomplishment. A critical question might be why the focal deep cooling of the colon is effective in protecting the spinal cord against SC-IRI. The main reason is likely the anatomic proximity of the descending colon and thoracolumbar spinal cord, such that cooling the tissue closer to this segment of the spinal cord will result in better protection against SC-IRI. Furthermore, previous studies have shown the potential negative consequences of aortic surgical procedures on intestinal ischemia,[19-22] consistent with the findings from the

present study (Fig 5). The intestinal ischemia could also be a likely early initiator of local and systemic inflammation[19,21] and, thus, worsening SC-IRI. Additionally, the major blood vessels to the distal thoracolumbar spinal cord are distributed in the area near the descending colon.[23,24] Therefore, the benefits from directly cooling the colon could be a combination of cooling the spinal cord, intestines, and nearby arterial vessels to the spinal cord in our animal model. Renal insufficiency is one of the serious complications after aortic clamping.[25] Although we did not measure blood renal injury biomarkers in the present study, in a similar balloon aortic occlusion study, the plasma level of creatinine was significantly increased at 180 minutes after aortic occlusion by approximately threefold of the preaortic occlusion level.[19] The increased creatinine level was significantly reduced by TRIC12°C cooling.[19] The result is consistent with the renal histologic changes shown in Fig 5. These findings might be clinically relevant, because aortic balloon occlusion is an integral part of the endovascular treatment of ruptured abdominal aortic aneurysms and noncompressible torso hemorrhage.[19-22] Bowel ischemia is a major complication of aortic balloon occlusion, which can be directly tied to increased operative time and blood loss.[19-22] Therefore, the use of the TRIC device could be a new tool to protect the bowel and spinal cord from ischemic injury in medical conditions in which aortic occlusion is required.

Study limitations. The present study had a few limitations. The spinal cord ischemia in the present study was induced by transient occlusion of the aorta, followed by reperfusion of all the intercostal branches. Therefore, this model might only partially replicate the reperfusion event after thoracoabdominal aneurysm repair in humans. Some intercostal branches to the spinal cord might not be reperfused by the thoracic endovascular aortic repair or open approach. Nonetheless, the use of the TRIC device has the potential to abrogate most of the major ischemia–reperfusion events after aortic clamping. This technique, because of its simplicity of use, could potentially be used in any clinical scenario in which temporary aortic clamping is needed.[1]

We noted that the rats in the TRIC12°C group had a significantly higher blood glucose level during the reperfusion phase. We hypothesized that this was likely the result of either a temporary decrease in tissue glucose consumption or reduced insulin sensitivity and insulin secretion under hypothermic conditions. The hyperglycemic effects on tissue IRI remain to be further evaluated. In clinical practice, this could be managed through intensive insulin therapy and frequent monitoring.[26]

It has generally been thought that cooling efficiency should be significantly lower in humans than in rats because of the different body mass. This might be true for skin cooling because the skin surface area/body mass (surface/mass) ratio is four times higher in rats than in humans, calculated using the Meeh formula (Appendix: surface/mass ratio calculation). In contrast, the colon surface/mass ratio is only ~1.3 times greater in rats than in humans. Therefore, using the surface/mass ratio, the TRIC focal cooling efficiency for abdominal organs might be only slightly lower in humans than in rats. This estimate has been supported by an ongoing study in our laboratory. That study demonstrated that directly cooling the gut using the TRIC device could also quickly reduce the intracolon temperature from 37°C to 10°C to 12°C within 10 minutes and bladder from 37°C to ~25°C within 30 to 40 minutes in a 45-kg naive swine, with maintenance of the esophageal upper body temperature in the tolerable range (Supplementary Fig 4).

CONCLUSIONS

Directly cooling the intestine focally with the TRIC device offered an exceptional survival rate and functional improvement after hypotensive aortic occlusion-induced spinal cord ischemia.

C.L. and D.Y. performed the animal model and data collection. R.C. and R.S. contributed to the research design, data analysis, data interpretation, and manuscript revision. B.H. designed the study and wrote the report and also contributed to the data analysis and data interpretation. Dr Charles Drucker was involved partially in the animal model and discussion of the study. Mr Chun Mun Loke, Mr Hironori Teramoto, and Ms Elizabeth Cote assisted with the data analysis, graphs, and references.

AUTHOR CONTRIBUTIONS

Conception and design: DY, CL, BH
Analysis and interpretation: YL, DY, RS, BH
Data collection: RSC, YL, DY, CL, RS, BH
Writing the article: RSC, YL, DY, BH
Critical revision of the article: RSC, CL, RS, BH
Final approval of the article: RSC, YL, DY, CL, RS, BH
Statistical analysis: RSC, DY, CL, RS, BH
Obtained funding: RSC, CL, RS, BH
Overall responsibility: BH
RC and YL contributed equally to this article and share co-first authorship.

REFERENCES

1. Liu C, Yuan D, Crawford R, Sarkar R, Hu B. Directly cooling gut prevents mortality in the rat model of REBOA management of lethal hemorrhage [e-pub ahead of print]. Shock, https://doi.org/10.1097/SHK.0000000000001744. Accessed January 22, 2021.
2. Qureshi AI, Afzal MR, Suri MFK. A population-based study of the incidence of acute spinal cord infarction. J Vasc Interv Neurol 2017;9:44-8.
3. Caton MT, Huff JS. Spinal Cord Ischemia. Treasure Island, FL: StatPearls; 2019.

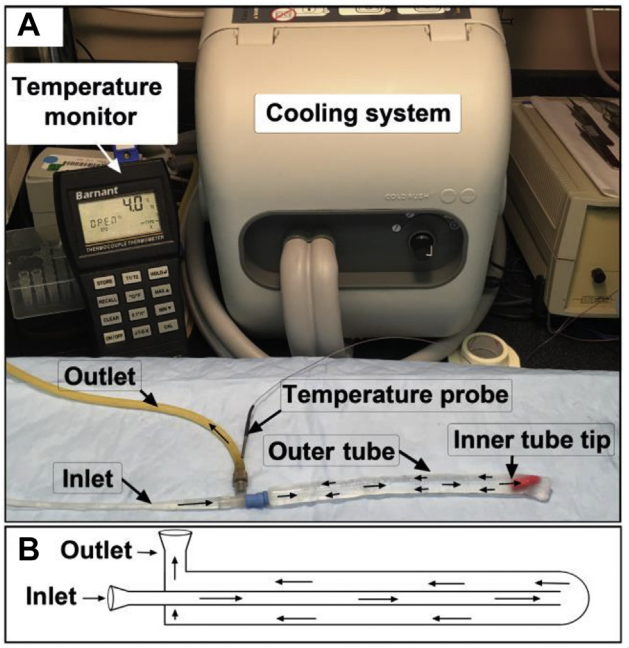
4. Sobel JD, Vartanian SM, Gasper WJ, Hiramoto JS, Chuter TA, Reilly LM. Lower extremity weakness after endovascular aneurysm repair with multibranched thoracoabdominal stent grafts. J Vasc Surg 2015;61:623-8.
5. Duggal N, Lach B. Selective vulnerability of the lumbosacral spinal cord after cardiac arrest and hypotension. Stroke 2002;33:116-21.
6. Weidauer S, Nichtweiss M, Hattingen E, Berkefeld J. Spinal cord ischemia: aetiology, clinical syndromes and imaging features. Neuroradiology 2015;57:241-57.
7. Alam HB, Chen Z, Li Y, Velmahos G, DeMoya M, Keller CE, et al. Profound hypothermia is superior to ultraprofound hypothermia in improving survival in a swine model of lethal injuries. Surgery 2006;140:307-14.
8. Taylor MJ, Bailes JE, Elrifai AM, Shih SR, Teeple E, Leavitt ML, et al. A new solution for life without blood: asanguineous low-flow perfusion of a whole-body perfusate during 3 hours of cardiac arrest and profound hypothermia. Circulation 1995;91:431-44.
9. Kheirbek T, Kochanek AR, Alam HB. Hypothermia in bleeding trauma: a friend or a foe? Scand J Trauma Resusc Emerg Med 2009;17:65.
10. Griepp RB, Di Luozzo G. Hypothermia for aortic surgery. J Thorac Cardiovasc Surg 2013;145(Suppl):S56-8.
11. Kanellopoulos GK, Kato H, Hsu CY, Kouchoukos NT. Spinal cord ischemic injury: development of a new model in the rat. Stroke 1997;28:2532-8.
12. Hwang JY, Sohn HM, Kim JH, Park S, Park JW, Lim MS, et al. Reproducible motor deficit following aortic occlusion in a rat model of spinal cord ischemia. J Vis Exp 2017;125:55814.
13. Prakash O, Jonson B, Bos E, Meij S, Hugenholtz PG, Hekman W. Cardiorespiratory and metabolic effects of profound hypothermia. Crit Care Med 1978;6:340-6.
14. Hess JR, Brohi K, Dutton RP, Hauser CJ, Holcomb JB, Kluger Y, et al. The coagulopathy of trauma: a review of mechanisms. J Trauma 2008;65:748-54.
15. Soreide K. Clinical and translational aspects of hypothermia in major trauma patients: from pathophysiology to prevention, prognosis and potential preservation. Injury 2014;45:647-54.
16. Moffatt SE, Mitchell SJB, Walke JL. Deep and profound hypothermia in haemorrhagic shock, friend or foe? A systematic review. J R Army Med Corps 2018;164:191-6.
17. Tisherman SA, Safar P, Radovsky A, Peitzman A, Marrone G, Kuboyama K, et al. Profound hypothermia (less than 10 degrees C) compared with deep hypothermia (15 degrees C) improves neurologic outcome in dogs after two hours' circulatory arrest induced to enable resuscitative surgery. J Trauma 1991;31:1051-61.
18. van der Worp HB, Macleod MR, Kollmar R; European Stroke Research Network for Hypothermia (EuroHYP). Therapeutic hypothermia for acute ischemic stroke: ready to start large randomized trials? J Cereb Blood Flow Metab 2010;30:1079-93.
19. Arya AK, Hu K, Subedi L, Li T, Hu B. Focal intra-colon cooling reduces organ injury and systemic inflammation after REBOA management of lethal hemorrhage in rats. Sci Rep 2021;11:13696.
20. Mehta M, Taggert J, Darling RC III, Chang BB, Kreienberg PB, Paty PS, et al. Establishing a protocol for endovascular treatment of ruptured abdominal aortic aneurysms: outcomes of a prospective analysis. J Vasc Surg 2006;44:1-8; discussion: 8.
21. Ultee KH, Zettervall SL, Soden PA, Darling J, Bertges DJ, Verhagen HJ, et al; Vascular Study Group of New England. Incidence of and risk factors for bowel ischemia after abdominal aortic aneurysm repair. J Vasc Surg 2016;64:1384-91.
22. Zhang J, Watson JD, Drucker C, Kalsi R, Crawford RS, Toursavadkohi SA, et al. Resuscitative endovascular balloon occlusion of the aorta (REBOA) not yet applicable for widespread out-of-hospital use: a case of nonsurvivable complication from prolonged REBOA inflation. Ann Vasc Surg 2019;56:354.e5-9.
23. Picone AL, Green RM, Ricotta JR, May AG, DeWeese JA. Spinal cord ischemia following operations on the abdominal aorta. J Vasc Surg 1986;3:94-103.
24. Jacobs MJ, de Mol BA, Elenbaas T, Mess WH, Kalkman CJ, Schurink GW, et al. Spinal cord blood supply in patients with thoracoabdominal aortic aneurysms. J Vasc Surg 2002;35:30-7.
25. Avgerinos DV, Payabyab EC, Rahouma M, Ruan Y, Gaudino M, Girardi LN. Elective proximal aortic surgery in patients with renal insufficiency. J Card Surg 2020;35:2194-200.
26. Forni AA, Rocchio MA, Szumita PM, Anger KE, Avery KR, Scirica BM. Evaluation of glucose management during therapeutic hypothermia at a tertiary academic medical center. Resuscitation 2015;89:64-9.

Submitted Apr 12, 2021; accepted Jul 27, 2021.

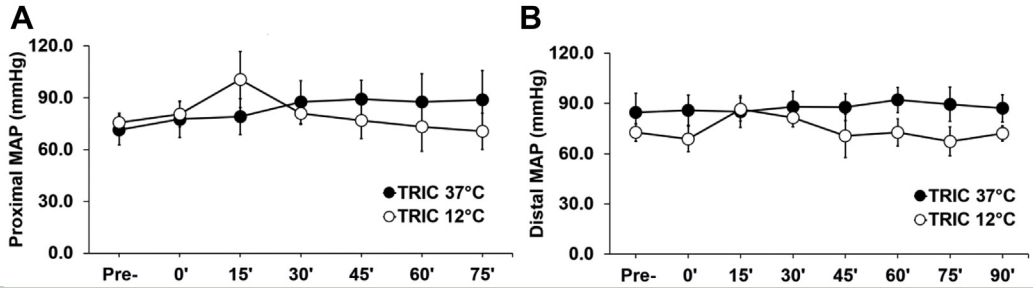
APPENDIX

Surface/Mass Ratio Calculation in Humans and RATS

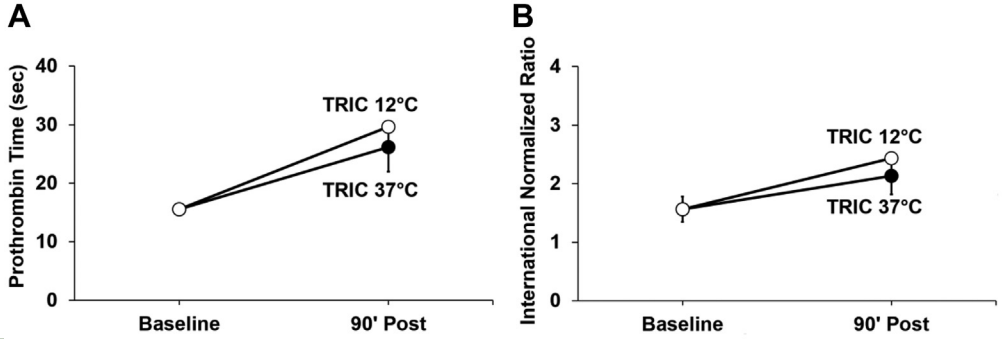
It has generally been thought that the cooling efficiency for a human body is significantly lower than that of a rat body because of the human's larger body mass. This might be true for skin cooling because the skin surface area/body mass (surface/mass) ratio is significantly smaller in humans than in rats. A 500-g rat's skin surface/mass is ~1.25. In contrast, the skin surface/mass of a 65,000-g and 175-cm height man is ~0.31, as calculated using the Meeh formula: surface area in cm^2 = kW 0.67 (k = 9.8 for rats and 12.3 for humans; W, body weight in grams). Thus, the skin surface/mass ratio is approximately four times greater for rats than for humans. Therefore, the skin cooling of a human body can be significantly more difficult relative to the skin cooling of a rat body. In contrast, the colon inner surface area of a 65,000-g and 175-cm man (5.5 cm in diameter and 120 cm in length) is ~2121 cm^2. Thus, the colon surface/mass ratio (2121/65,000) is ~0.032. In contrast, the colon surface area of a 500-g rat (0.6 cm in diameter and 11 cm in length) is ~21 cm^2, with a ratio (21 of 500) of ~0.042. Thus, the colon surface/mass ratio is only ~1.3 times higher in rats than in humans, significantly smaller than the difference in the skin surface/mass ratio (four times) between humans and rats. Therefore, based on the colon surface/mass ratio, the TRIC focal cooling efficiency for abdominal organs might be only slightly lower in humans than in rats.



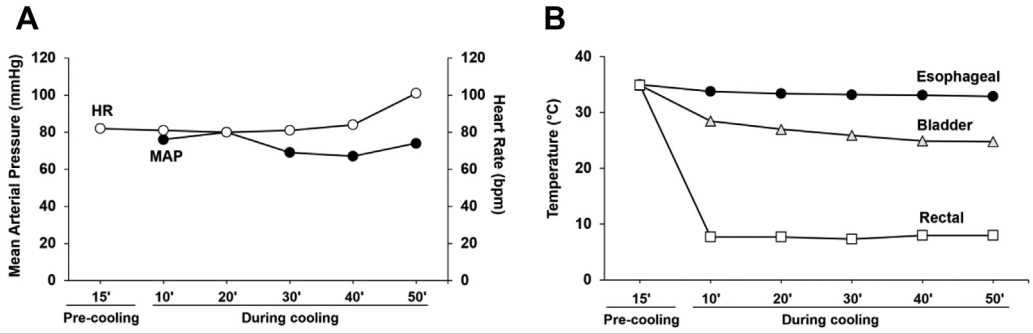
Supplementary Fig 1. A, Transrectal intracolon (TRIC) temperature management device. The TRIC device consists of a two-lumen catheter with an inflow tube and an outflow external membrane, temperature sensors and monitors, and a cooling fluid reservoir with a circulator pump (cooling system). Two temperature sensors (model HYP-3; Omega Engineering, Norwalk, Conn) were placed inside the "inlet" and "outlet" for recording the heat exchange between the colorectal segment of the catheter. **B,** Schematic of the TRIC circuit. *Arrows* denote the flow directions. The TRIC device circulates a cold water or saline solution within a conformable catheter that maximizes the surface contact with the rectal and colon wall, ensuring effective thermal transmission to the adjoining visceral tissues. When not filled or inflated by circulating fluid, this device is thin and relatively fixable and, thus, can be easily inserted into the rectum and further advanced into the descending colon. Once inflated by a low-pressure circulating fluid, the TRIC device apposes the rectal and colon inner surface; thus, it can cool the rat abdominal cavity to 10°C in <5 minutes. Using a closed-circuit system eliminates the risk of electrolyte abnormalities associated with colonic or gastric lavage. The rectal route bypasses the skin and subcutaneous fat that evolutionarily developed to be highly resistant to heat transfer, and the lack of high-volume regional blood flow during balloon occlusion of the aorta ensures that inadvertent systemic cooling is minimized. The large surface area of the colon and its immediate proximity to the spinal cord means that rapid thermal conduction can be effected. These features offer critical advantages for the TRIC strategy to prevent abdominal organ and spinal cord injury during and after aortic repair surgery.



Supplementary Fig 2. Recordings of the proximal **(A)** and distal **(B)** mean arterial pressure (MAP). The rats were subjected to sham surgery followed by recovery. Data presented as the mean ± standard error of the mean. No statistically significant differences were present in the proximal and distal MAPs between the transrectal intracolon (TRIC) device at 12°C and 37°C (TRIC12°C and TRIC37°C, respectively) groups. One-way analysis of variance, followed by the Tukey test, was used for statistical analysis.



Supplementary Fig 3. Effects of transrectal intracolon (TRIC) cooling on the prothrombin time (PT; **A**) and international normalized ratio (INR; **B**). The PT/INR were measured via the Coag-Sense PT/INR Professional System (CoaguSense, Inc, Fremont, Calif) at 10 minutes before surgery (baseline) and 90 minutes after sham surgery in the TRIC at 12°C and 37°C (TRIC12°C and TRIC37°C, respectively) groups. The increase in PT/INR at 90 minutes postoperatively likely resulted from the use of heparin during the surgical procedure (see the Methods section). Data presented as the mean ± standard error of the mean. No significant differences were found between the TRIC12°C and TRIC37°C groups (two-sided *t* test).



Supplementary Fig 4. Transrectal intracolon (TRIC) device at 12°C (TRIC12°C) temperature management in a naive swine. **A,** Heart rate and mean blood pressure (MAP). **B,** Esophageal, bladder, and rectal temperatures. A 45-kg swine was anesthetized with isoflurane and underwent TRIC12°C cooling for the period indicated. The heart rate and MAP and esophageal, bladder, and rectal temperatures were recorded throughout the TRIC12°C cooling period. The data demonstrated that under normal circulation conditions (normal heart rate and blood pressure; **A**), TRIC cooling could quickly reduce the intracolon temperature from 37°C to 10°C within 10 minutes and bladder temperature from 37°C to 25°C within ~30 minutes in a 45-kg naive swine, with maintenance of the esophageal upper body temperature in the tolerable range. The focal cooling efficiency should be further and significantly increased when aortic occlusion occurs because the normal circulation will remove the cold temperature away from focal cooling through heat exchange.