

# Fast Hypothermia Induced by Continuous Renal Replacement Therapy Alleviates Renal and Intestinal Injury After Cardiac Arrest in Swine

**Jiefeng Xu**

Second Affiliated Hospital, Zhejiang University School of Medicine

**Lin Shi**

Second Affiliated Hospital, Zhejiang University School of Medicine

**Jiangang Wang**

Hangzhou Emergency Medical Centre

**Chunshuang Wu**

Second Affiliated Hospital, Zhejiang University School of Medicine

**Qijiang Chen**

The First Hospital of Ninghai

**Xiaohong Jin**

The First People's Hospital of Wenling

**Jungen Zhang**

Hangzhou Emergency Medical Centre

**Mao Zhang** (✉ [z2jzk@zju.edu.cn](mailto:z2jzk@zju.edu.cn))

Zhejiang University School of Medicine Second Affiliated Hospital <https://orcid.org/0000-0001-7919-8213>

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## Original research

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# Abstract

## Background:

Renal and intestinal damage lead to multiple organ dysfunction and death after cardiopulmonary resuscitation (CPR), and can be partly mitigated by therapeutic hypothermia. Currently, continuous renal replacement therapy (CRRT) was demonstrated to be an effective way to induce hypothermia. In the present study, we aimed to investigate the influence of CRRT cooling on renal and intestinal damage after CPR based on a porcine model.

## Methods:

32 swine were subjected to ventricular fibrillation for 8 min, while defibrillation was performed at 5 min of CPR. All pigs were randomly allocated to receive CRRT (n = 9), surface cooling (SC, n = 9), normothermia (NT, n = 9) or sham control (Control, n = 5) at 5 min post resuscitation. In the CRRT group, the pigs were cooled by the combination of 8-hr CRRT and 16-hr SC, a rate of 180 ml/min of blood flow was initially set with the infusion line submerged in 4 °C of ice water. In the SC group, pigs were cooled by the 24-hr SC. As to the NT and Control groups, the temperatures were maintained at a normal range.

The levels of creatinine (Cr), blood urea nitrogen (BUN), intestinal fatty acid binding protein (IFABP) and diamine oxidase (DAO) in serum were measured at baseline and at 1, 3, 6, 12, 24 and 30 h post resuscitation. Additionally, tissues of kidney and intestine were harvested, from which the levels of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-6 (IL-6), the contents of malondialdehyde (MDA), activities of superoxide dismutase (SOD) and the apoptosis levels were analyzed.

## Results:

After resuscitation, the blood temperature decreased significantly more rapidly in the CRRT group than in the SC group ( $9.8 \pm 1.6$  vs.  $1.5 \pm 0.4$  °C/h,  $P < 0.01$ ). The levels of Cr, BUN, IFABP and DAO after resuscitation were significantly lower in the two hypothermic groups compared with the NT group. Furthermore, from pathological evidence, cooling induced by CRRT alleviated post-resuscitation renal and intestinal injury compared to SC.

## Conclusion:

Fast hypothermia induced by continuous renal replacement therapy was superior to surface cooling in mitigating renal and intestinal injury post resuscitation.

## Background

In resuscitated patients undergone cardiac arrest, the systemic ischemia-reperfusion (I/R) injury often induces a sepsis-like syndrome or post-cardiac arrest syndrome (PCAS), leading to multiple organ injuries with brain, heart, kidney and intestine, etc, involved in [1]. Several attempts have been made to mitigate the

brain and heart injury after resuscitation, which have been considered as main causes of morbidity and mortality for cardiac arrest (CA) survivors, yet renal and intestinal injury have received less attention. In fact, almost 50% of CA survivors suffer acute kidney injury (AKI), and nearly 16% are treated with renal replacement therapy[2]. AKI occurs owing to a combination of I/R injury and hypoperfusion due to circulatory shock following resuscitation[2]. Post-arrest AKI is strongly related with worse neurological outcome and survival[2-4]. Besides, nearly 60% of survivors of CA are presented with intestinal injury[5], which has been proved via endoscopy and autopsy after CPR[5, 6]. Due to the susceptibility to I/R, mesenteric ischemia often occurs, causing an increase in intestinal permeability and subsequent bacterial translocation[7], which may be exceedingly detrimental[8]. Additionally, the elevation of biomarkers of intestinal injury such as fatty-acid binding protein (IFABP) after CA are related with endotoxemia, further worsening post-resuscitation organ failure and shock[9].

Therapeutic hypothermia (TH) has been a main therapeutic intervention of post-resuscitation care. The protective role of TH on cardiac dysfunction and brain injury has been studied previously, but its influence on renal and intestinal damage was not intensively investigated yet. A previous study demonstrated that 24 hr of TH after CPR was associated with a delayed improvement in renal function[10]. Hasslacher et al[11] showed that TH had a protective effect against the development and recovery of AKI, accompanied by lower levels of creatinine (Cr) and cystatin C. With regard to the role of TH on intestinal injury, a study showed TH alone or in the combination with sevoflurane could affect small intestinal protein expression and activity, and the proteins might be involved in intestinal I/R injury following resuscitation[12]. Another study showed that ulinastatin combined with TH might exert a protective effect in the small intestinal mucosa by decreasing oxidative stress in a rat model[13].

However, despite TH has been proved to exert potent protection for CA survivors[14, 15], more than 50% of them die or have poor neurological outcome[16-18]. A review demonstrated that rapid cooling exerted a higher rate of good neurological outcome than slower cooling methods[19]. A cooling rate of  $> 3^{\circ}\text{C}/\text{h}$  appear to be beneficial to arrive at a targeted temperature below  $34^{\circ}\text{C}$  within 3.5 h after return of spontaneous circulation (ROSC)[19]. Therefore, the undesirable outcome of TH may be deprived from the unsatisfactory cooling effects of traditional methods such as surface blanket cooling, rapid infusion of ice lactated Ringer's solution, etc. Previous studies noted that conventional cooling methods usually take several hours to decrease the body temperature[20]. Hence, rapid cooling may benefit resuscitated patients more than slower cooling methods. Previous research has attempted to apply several techniques to induce fast hypothermia, including liquid ventilation[21, 22], peritoneal lavage system[23] and intravenous cooling[24], yet it's hard to incorporate them into the clinical setting due to the required specific equipment.

Recently, continuous renal replacement therapy (CRRT) can also be used as another type of cooling method. Karacan et al[25] and Ma et al[26] have reported case reports on using CRRT to induce and maintain TH. Du et al[27] showed the combination of CRRT and TH enabled acute heart failure to achieve recovery of cardiac function. CRRT, as a common and well-developed treatment in critical ill patients, will be a potent cooling method for CA survivors. Recently, we have demonstrated the fast hypothermia

induced by CRRT had protective effects in systemic inflammation, heart and brain damage after resuscitation in a porcine model[28].

However, the influence of hypothermia induced by CRRT on renal and intestinal dysfunction hasn't been determined. The aim of this research was to investigate the influence of CRRT cooling on renal and intestinal injury following CPR in swine.

## Methods

This study was a randomized, controlled laboratory experiment based on a pig model of cardiac arrest. All the experimental procedures were performed based on the methods of our one previous study, in which the animal model has been well established[29]. Ethics committee approval was obtained from the Second Affiliated Hospital, Zhejiang University School of Medicine (No. 2019004). The study included 32 healthy male domestic pigs purchased from same vendor (Shanghai Jiagan Biotechnology Inc., Shanghai, China), weighting  $36 \pm 2$  kg. The pigs were housed under controlled pressure, temperature, humidity and lighting conditions. They were given water and food regularly, washed regularly and disinfected in closed cages. All pigs received care based on the Principles of Laboratory Animal Care and the Guide for the Care and Use of Laboratory Animals.

### Animal preparation

The night before the experiment food was withdrawn from all animals, but they were available to water intake. Induction of anesthesia in pigs was achieved by a combination of intramuscular ketamine injection at 20 mg/kg and sodium pentobarbital injection at 30 mg/kg in an ear vein. Then, to maintain the anesthesia, sodium pentobarbital at 8 mg/kg/h as well as fentanyl at 2 mg/kg/h were given intravenously. Ventilation was maintained using a volume-controlled ventilator (SynoVent E5, Mindray, Shenzhen, China) with the following setting: tidal volume, 12 mL/kg; peak flow, 40 L/min and  $FiO_2$ , 21%. End-tidal carbon dioxide (ETCO<sub>2</sub>) was measured using an ETCO<sub>2</sub>/SPO<sub>2</sub> monitor (PMSH-300, SunLife Science Inc., Shanghai, China), maintaining at 35 mmHg ~ 40 mmHg by respiratory frequency. The standard lead II electrocardiogram surface electrode was secured.

A double-lumen catheter (11 F, Gambro Katheter technik Hechingen, Hechingen, Germany) was placed into the left femoral vein to establish vascular access of CRRT. A fluid-filled catheter (8 Fr, C.R. Bard Inc., Salt Lake, UT) was placed via the right femoral artery to the thoracic artery to measure aortic pressure. A thermodilution catheter (7 Fr, Abbott Critical Care # 41216, Chicago, IL) was placed via right femoral vein to the right atrium to monitor right atrial pressure and blood temperature. Intermittent heparinised saline flushes were made to avoid clogging of the catheters. A pacing catheter (5 F, EP Technologies Inc., Mountain View, CA) was placed via the right external jugular vein to the right ventricle to induce ventricular fibrillation (VF). Animals were placed supine on a heating blanket to maintain the normal temperature at  $38.0 \pm 0.5^\circ\text{C}$ .

### Experimental protocol

Baseline characteristics were recorded after a 10-min stabilization. Pigs were randomly allocated to 1 of 4 groups: normothermia (NT, n=9), surface cooling (SC, n=9), CRRT cooling (CRRT, n=9), or sham control (Control, n = 5). Pigs in the NT and Control groups had the body temperature maintained at  $38.0 \pm 0.5^{\circ}\text{C}$  using the Blanketrol III (Cincinnati Sub-Zero, Cincinnati, OH). For pigs in the other 2 groups, TH was started at 5 min after CPR, and then maintained at the temperature of  $33 \pm 0.5^{\circ}\text{C}$ . The hypothermia induced in the CRRT group was achieved by 8-hr CRRT, using an AN69ST hemofilter (Gambro Industries Inc., Meyzieu, France), followed by 16-hr SC; while the cooling in the SC group was achieved by 24-hr SC with the Blanketrol III. Then, a  $1^{\circ}\text{C}/\text{h}$  rate of rewarming were followed.

For the CRRT group, a 180 ml/min rate of the blood flow was determined initially, immersing the circuit in  $4^{\circ}\text{C}$  ice water until the target temperature of  $33^{\circ}\text{C}$  arrived. Then, the temperature was maintained and the blood flow reduced by 60 mL/min. The rates of liquid replacement and ultrafiltration were 30 mL/kg/h and 20 mL/kg/h, respectively. Immediately when CRRT started, a load dose of 1000-IU heparin was given for anticoagulation, followed by a dose of 150 IU, 300 IU, 450 IU for the first three hours, respectively, and 600 IU/h for the rest 5 hours.

For all pigs, we induced VF by delivering an alternating current of 1 mA. Anesthesia and ventilation were disconnected, and the animals underwent an 8-min period of untreated VF. Then, cardiac pulmonary resuscitation (CPR) was started, with a ratio of 30:2 of compression to ventilation. The chest compressions were achieved at a rate of 100 ~ 120/min (reaching 50 ~ 60mm deep) by a monitor defibrillator (ZOLL Medical Inc., Chelmsford, MA). A dose of 20mg/kg epinephrine was administered at 2.5 min during resuscitation. Biphasic defibrillation at 150 J was performed at 5min of CPR. ROSC was determined as an organized rhythm and mean arterial pressure of  $>50$  mmHg sustaining for  $> 5$  min. If not achieved, CPR was held on for another 2 min prior to the next defibrillation. This cycle was duplicated every 2 min, and administration of epinephrine was carried out every 3 min until successful ROSC or 15 min had elapsed. If achieved, 30-hr mechanical ventilation and infusion of normal saline were subsequently continued to keep fluid balance. After completion of the study, euthanasia and a following necropsy were executed to confirm potential injuries of thoracic or abdominal viscera due to experimental intervention. The experimental flow diagram was shown in Fig. 1A.

## Measurements

Blood samples of veins and arteries were collected at 1, 3, 6, 12, 24, and 30h post resuscitation. Then the researchers separated serums from venous blood samples and stored them at  $-80^{\circ}\text{C}$  prior to further analyzing the levels of creatinine (Cr), blood urea nitrogen (BUN), intestinal fatty acid binding protein (IFABP) and diamine oxidase (DAO). To evaluate the inflammatory response and oxidative stress, kidney and intestine tissues from renal parenchyma and middle part of small intestine were harvested immediately after euthanasia, and subsequently frozen in liquid nitrogen prior to analysis. Levels of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-6 (IL-6) were analyzed using enzyme-linked immunosorbent assay kits (ELISA, Meixuan Biotechnology Inc., Shanghai, China). The contents of malondialdehyde (MDA) were measured by thiobarbituric acid reactive substances assay, and activities of superoxide dismutase

(SOD) were measured by xanthine oxidase assay [30] (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

The extent of apoptosis of the kidney and intestine were measured using terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) assay. The proportion of apoptotic cells was determined as the percentage of TUNEL-positive cells/total cells, and the cleaved caspase-3 protein was detected using immunohistochemistry. The staining intensity of cleaved caspase-3-positive undergone semiquantitative analysis through integrated optical density based on a previous study [31] with Image-Pro Plus 6.0 software (Media Cybernetics, Silver Spring, MD).

## Statistical analysis

Continuous variables were described as mean  $\pm$  standard deviation (SD) or median (25th~75th percentiles) for data normally distributed or not. For comparisons among multiple groups, one-way analysis of variance was used for data normally distributed, Kruskal–Wallis test for data not normally distributed. Bonferroni test was used to account for any two group comparisons when the overall comparison was significant. Categorical data were analyzed using Fisher exact test. A two-sided *P* value of  $<0.05$  was considered as statistically significant.

## Results

A total of 32 pigs went through experiments completely, baseline characteristics and chemistries among all groups were mathematically the same (Fig. 1B). During CPR period, animals that experienced CA and resuscitation had an even level of CPP. In all three groups, 8/9 animals achieved ROSC, respectively. There was no significant difference as to coronary perfusion pressure (CPP), CPR duration, times of defibrillation, epinephrine dosage among 3 groups (Fig. 2A). During post resuscitation period, the temperature in the CRRT group decreased significantly faster than in the SC group ( $9.8 \pm 1.6$  vs.  $1.5 \pm 0.4$  °C/h,  $P < 0.01$ , Fig. 2B).

During post resuscitation period, the values of creatinine (Cr) showed tendency to descend in two hypothermic groups. At 6h, 12h and 24h after resuscitation, compared to the NT group, the serum levels of Cr were significantly lower in two hypothermic groups, with the lowest in the CRRT group (all  $P < 0.05$ ). In addition, the levels of blood urea nitrogen (BUN) in all groups increased in the first 24h-post resuscitation but decreased in the remaining 6h-rewarming period. Compared to the NT group, the levels of BUN were significantly lower in the two hypothermic groups, with the lowest in the CRRT group at 6h, 12h, 24h and 30h post resuscitation (all  $P < 0.05$ ). Conclusively, in the 24h-post resuscitation period, compared with the SC and NT group, renal injury in the CRRT group was alleviated (all  $P < 0.05$ , Fig. 3).

In the tissues of kidney, compared with the NT group, the levels of TNF- $\alpha$ , IL-6 were much lower in two hypothermic groups, with the lowest in the CRRT group (all  $P < 0.05$ ). With regard to the effect on oxidative stress, the lowest level of MDA and the highest level of SOD were observed in the CRRT group among three experiment groups ( $P < 0.05$ ). In the same way, the proportions of apoptotic cells and the

expressions of cleaved caspase-3 in the kidney of the two hypothermic groups were lower than the NT group, with the lowest in the CRRT group ( $P < 0.05$ ). Conclusively, the cooling induced by CRRT alleviated the tissue inflammation, oxidative stress as well as the extent of apoptosis in the kidney compared with SC ( $P < 0.05$ , Fig. 4).

Intestinal status was assessed using the levels of IFABP and DAO. After resuscitation, the values of IFABP increased in the first 24h and decreased in the remaining 6h-rewarming period. At 24h after resuscitation, the serum levels of IFABP were the lowest in CRRT group, followed by the SC group and the NT group ( $P < 0.05$ ). In addition, the levels of DAO in all groups showed tendency to ascend in the first 12h post resuscitation, followed to descend in the remaining 18h post resuscitation. Compared to the NT group, the levels of DAO were significantly lower in two hypothermic groups, with the lowest in the CRRT group at 12h, 24h and 30h post resuscitation (all  $P < 0.05$ ). Conclusively, in the 24h-post resuscitation period, compared with the SC and NT group, intestinal injury was significantly alleviated in the CRRT group (all  $P < 0.05$ , Fig. 5).

In the tissues of intestine, compared with the NT group, the levels of TNF- $\alpha$ , IL-6 were much lower in the two hypothermic groups, with the lowest in the CRRT group (all  $P < 0.05$ ). With regard to the effect on oxidative stress, the lowest level of MDA and the highest level of SOD were observed in the CRRT group among the three experiment groups ( $P < 0.05$ ). In the same way, the proportions of apoptotic cells and the expression of cleaved caspase-3 in the intestine in the two hypothermic groups were lower than in the NT group, with the lowest in the CRRT group ( $P < 0.05$ ). Conclusively, compared with SC, cooling induced by CRRT mitigated the tissue inflammation, oxidative stress and apoptosis of cells in the intestine ( $P < 0.05$ , Fig. 6).

## Discussion

This present study focused on the potential organ protection of CRRT cooling against PCAS based on a swine model. We found the protective effect of CRRT cooling was significantly superior to SC from the following aspects: 1) hypothermia induced by CRRT was achieved significantly faster than SC; 2) CRRT cooling significantly alleviated the renal and intestinal injury post resuscitation compared to SC; 3) with pathologic evidence, the protective effect of CRRT cooling on kidney and intestine were exerted by decreasing the inflammation of tissues, suppressing oxidative stress and decreasing the extent of apoptosis compared with SC.

Laurent et al [32] found that the combination of CRRT with TH was feasible in CA patients, and might improve the prognosis of PCAS. Two cases reports demonstrated that CRRT cooling yielded better neurological recovery for CA and acute heart failure [26, 27]. Therefore, cooling induced by CRRT might offer an efficient way to produce TH in the clinical practice. Based on a previous study, 8-hr CRRT cooling followed by 16-hr SC was determined in our study to reduce the possibility of adverse events [32]. And no adverse events were observed in the CRRT group. Thus, CRRT cooling might be a safe way to induce TH with high efficiency. Additionally, a review [19] demonstrated that the achievement of temperature below

34°C within 3.5h after ROSC seemed to be beneficial. In the present study, the blood temperature was initially decreased rapidly by submerging the circuit in ice water to release heat, followed by the maintenance of TH by wrapping the circuit within an adjustable heating device.

Most importantly, the present study found that rapid therapeutic hypothermia induced by CRRT significantly mitigated the renal and intestinal injury after resuscitation with comparison with SC. Cr and BUN are two well-recognized markers of renal function. Previous review showed that higher Cr levels on admission was a significant predictor of AKI occurrence[2]. The IFABP is a cytosolic protein, which will be released into the circulation when intestinal permeability increases, making it an efficient marker for intestinal damage[33]. Evidence has shown a linear relation between max endotoxin level and max IFABP ( $p = 0.01$ ), suggesting that the higher the intestinal injury, the higher the level of endotoxin[9]. And DAO is an intracellular enzyme mainly located in intestinal mucosa, catalyzing oxidative deamination of diamines[34]. Recent evidence suggests that the rise in plasma DAO level is strongly associated with the severity of intestinal tissue damage[35]. Therefore, the activity of plasma DAO can manifest the intestinal status and will increase in case of intestinal ischemia[34]. In our study, with a smaller increase of levels of Cr, BUN, IFABP and DAO, potential renal and intestinal protection by CRRT cooling was proved.

Evidence from pathologic processes, including decreasing the inflammation of tissues, suppressing oxidative stress and decreasing the level of apoptosis, was observed in tissues of kidney and intestine in the CRRT group. According to a growing body of literature, TNF- $\alpha$  and IL-6 are typical indexes to manifest the inflammatory response of the whole body or certain organs[36]. With a smaller elevation of TNF- $\alpha$  and IL-6, CRRT cooling might have a protective influence by decreasing tissue inflammation in the kidney and small intestine. And the levels of MDA and SOD in tissues were also determined in our study. MDA, a substance produced by reactive oxygen after degrading polyunsaturated lipids and may lead to intracellular toxic stress, is an indicator of oxidative stress, while SOD is a pivotal antioxidant in cells[13]. With a smaller elevation of MDA and larger elevation of SOD, CRRT cooling may alleviate the renal and intestinal injury by suppressing oxidative stress. Previous studies found that CRRT could not decrease systemic inflammation after resuscitation in both CA patients[32] and rat models[37]. Therefore, the organ protective effects in our study might be mostly ascribed to the fast induction of hypothermia by CRRT cooling.

In the kidney and intestine among 3 experiment groups, the lowest proportions of apoptotic cells and lowest expression of cleaved caspase-3 were observed in CRRT group ( $P < 0.05$ ), which contribute to strengthening the protective effect by hypothermia induced by CRRT on renal and intestinal injury. Caspase-3 is required for most of the proteolysis during apoptosis, and the cleaved caspase-3 has been used to represent the level of cell apoptosis[38]. Therefore, we can conclude that CRRT cooling might exert the organ protective effect by inhibiting the process of apoptosis.

There were several limitations that should be stated. Firstly, a 1°C/h rate of rewarming was used in our study based on previous results[39], while rewarming should be achieved at rate of about 0.25 ~ 0.5°C per hour based on the current guideline[40]. Second, the apparatus for cooling induced by CRRT was ready in

advance, so the induction of TH was started immediately ROSC was obtained. However, a longer duration of more than 15 min are required to perform CRRT in the clinical setting. Third, heparin was used as the anticoagulant in CRRT in our study, while the use of citrate is preferred as recommended in the Kidney Disease Improving Global (KDIGO) clinical practice because of its high efficacy and safety[41]. A higher risk of bleeding exists when heparin is used[42].

## Conclusions

Fast hypothermia induced by continuous renal replacement therapy was superior to surface cooling in mitigating renal and intestinal injury postresuscitation.

## Abbreviations

AKI: Acute kidney injury; BUN: Blood urea nitrogen; CA: Cardiac arrest; CPP: Coronary perfusion pressure; CPR: Cardiac pulmonary resuscitation; Cr: Creatinine; CRRT: Continuous renal replacement therapy; DAO: Diamine oxidase; ELISA: Enzyme-linked immunosorbent assay kits; ET $\text{CO}_2$ : End-tidal carbon dioxide; IFABP: Intestinal fatty acid binding protein; IL-6: Interleukin-6; I/R: Ischemia-reperfusion; KDIGO: Kidney Disease Improving Global; MDA: Malondialdehyde; NT: Normothermia; PCAS: Post-cardiac arrest syndrome; ROSC: return of spontaneous circulation; SC: Surface cooling; SD: Standard deviation; SOD: Superoxide dismutase; TH: Therapeutic hypothermia; TNF- $\alpha$ : Tumor necrosis factor- $\alpha$ ; TUNEL: Terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling; VF: Ventricular fibrillation.

## Declarations

### Ethics approval and consent to participate

The approval from the Ethics Committee of the Second Affiliated Hospital of Zhejiang University School of Medicine was obtained (approval number: 2019004).

### Consent for publication

Not applicable.

### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Competing interests

The authors declare that they have no competing interests.

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### Authors' contributions

MZ, JGZ contributed substantially to the conception and design of the work. JFX, LS, JGW, CSW, QJC, XHJ conducted experiments. LS, JFX contributed to the analysis and interpretation of data. LS, JFX drafted the original manuscript, and MZ, JGZ revised the manuscript. All authors read and approved the final manuscript.

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### Authors' information

<sup>1</sup>Department of Emergency Medicine, Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310009, China; <sup>2</sup>Institute of Emergency Medicine, Zhejiang University, Hangzhou 310009, China; <sup>3</sup>Hangzhou Emergency Medical Centre, Hangzhou 310021, China; <sup>4</sup>Department of Intensive Care Medicine, The First Hospital of Ninghai, Ningbo 315000, China; <sup>5</sup>Department of Emergency Medicine, The First People's Hospital of Wenling, Taizhou 317500, China<sup>3,†</sup>. Jiefeng Xu, Lin Shi contributed equally to this work, and should be regarded as co-first authors.

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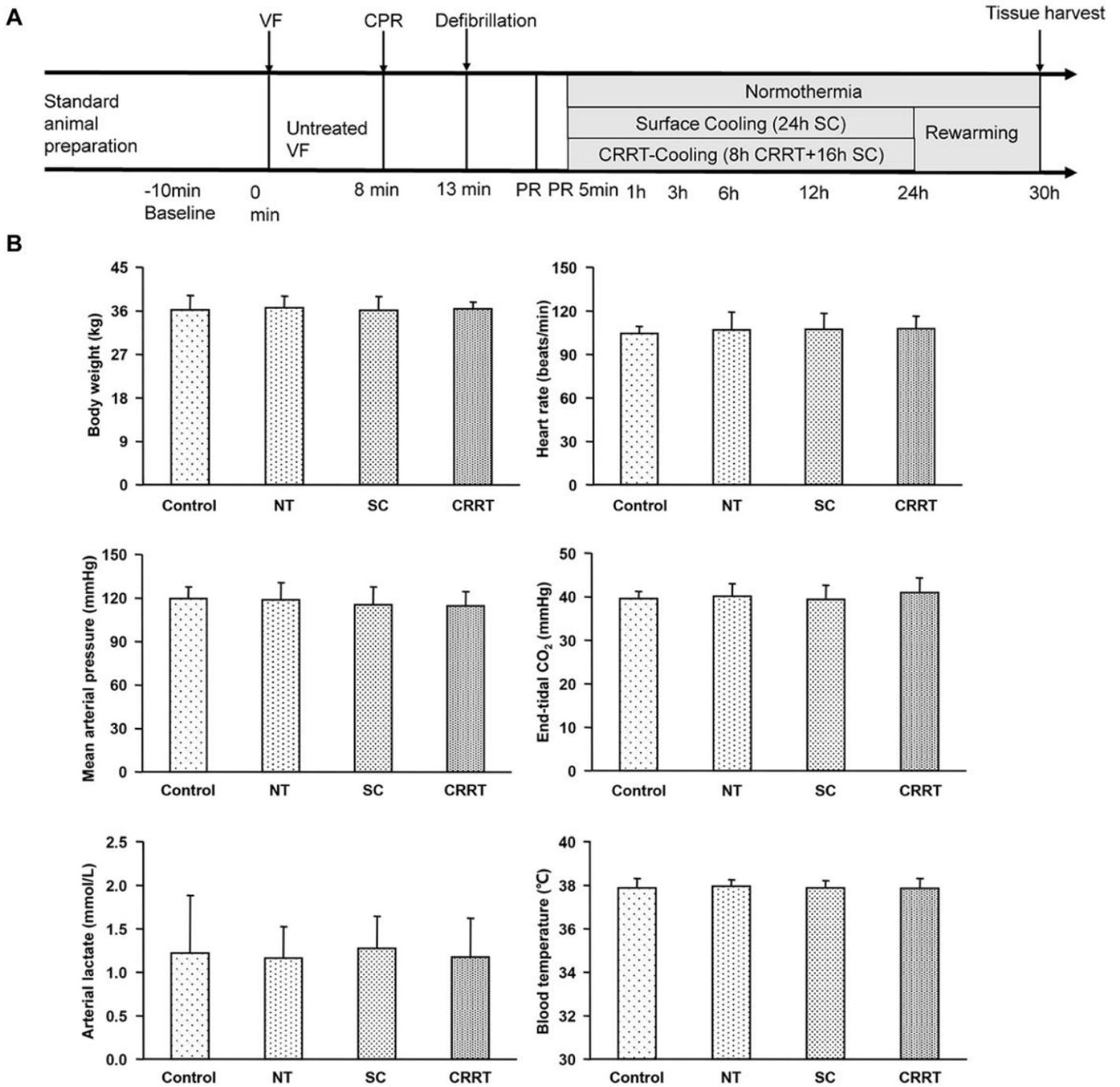
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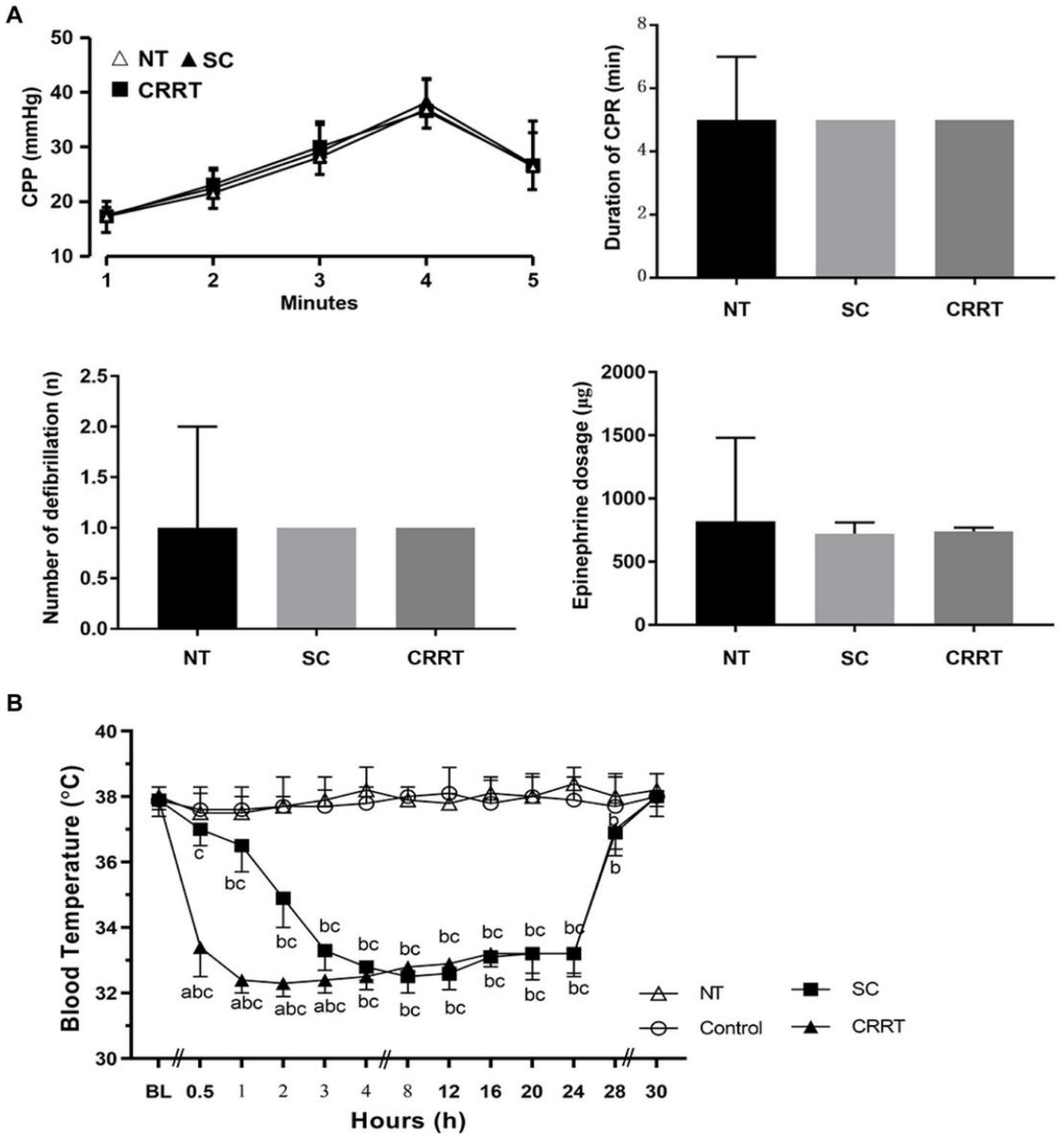
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## Figures



**Figure 1**

Experimental procedures and baseline data of all animals. A, Experimental procedures; B, Baseline data of all animal. CPR indicates cardiac pulmonary resuscitation; CRRT, continuous renal replacement therapy; NT, normothermia; PR, post resuscitation; SC, surface cooling; VF, ventricular fibrillation.



**Figure 2**

Cardiopulmonary resuscitation outcomes and the changes of blood temperature of three groups (note: except the control group containing 5 swine, the other groups have 9 swine each). A, Cardiopulmonary resuscitation outcomes; B, The changes of blood temperature. BL indicates baseline; CPP, coronary perfusion pressure; CPR, cardiac pulmonary resuscitation; CRRT, continuous renal replacement therapy

cooling; NT, normothermia; PR, post resuscitation; SC, surface cooling. aP< 0.05 versus SC group; bP< 0.05 versus NT group; cP< 0.05 versus Control group.

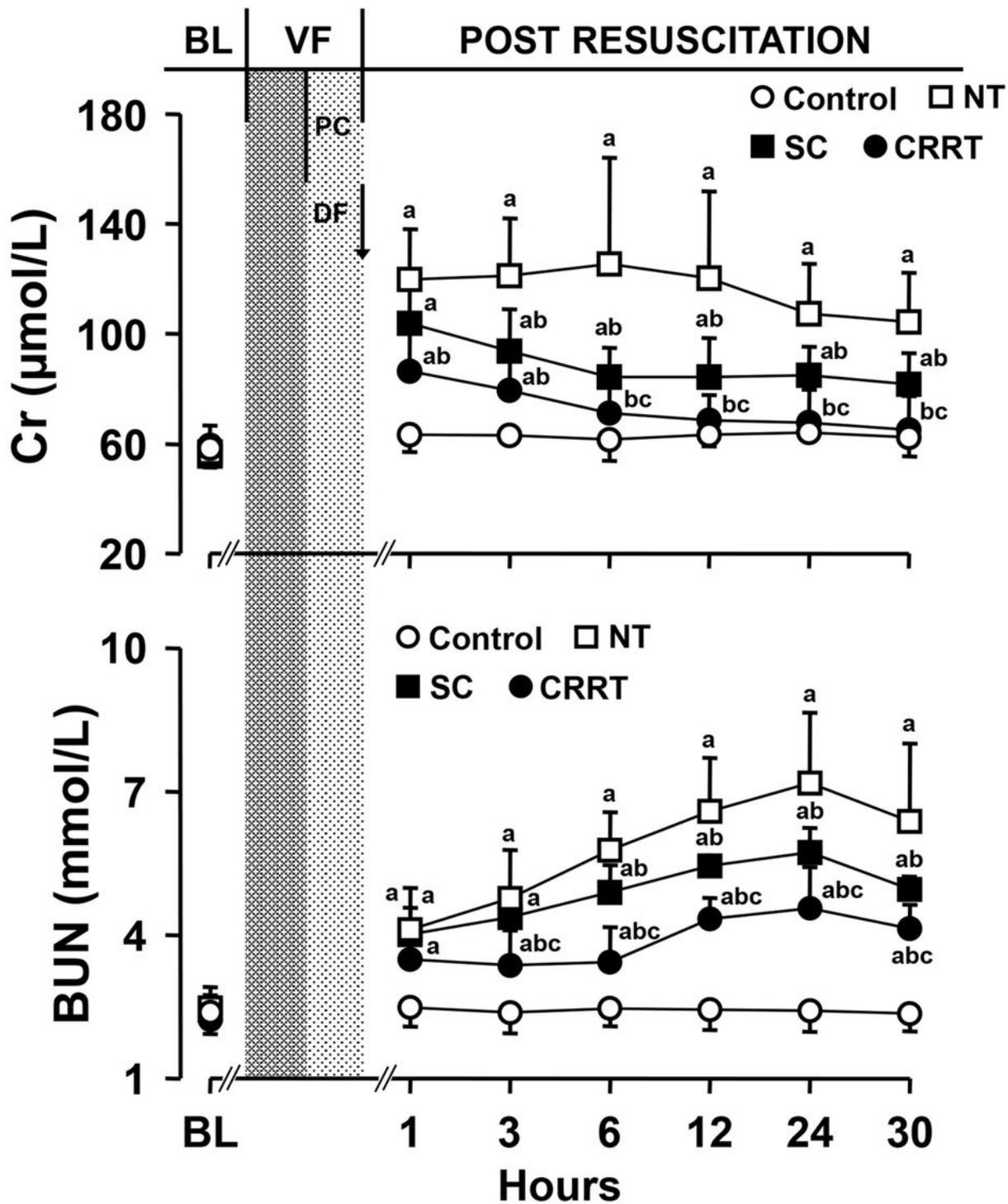
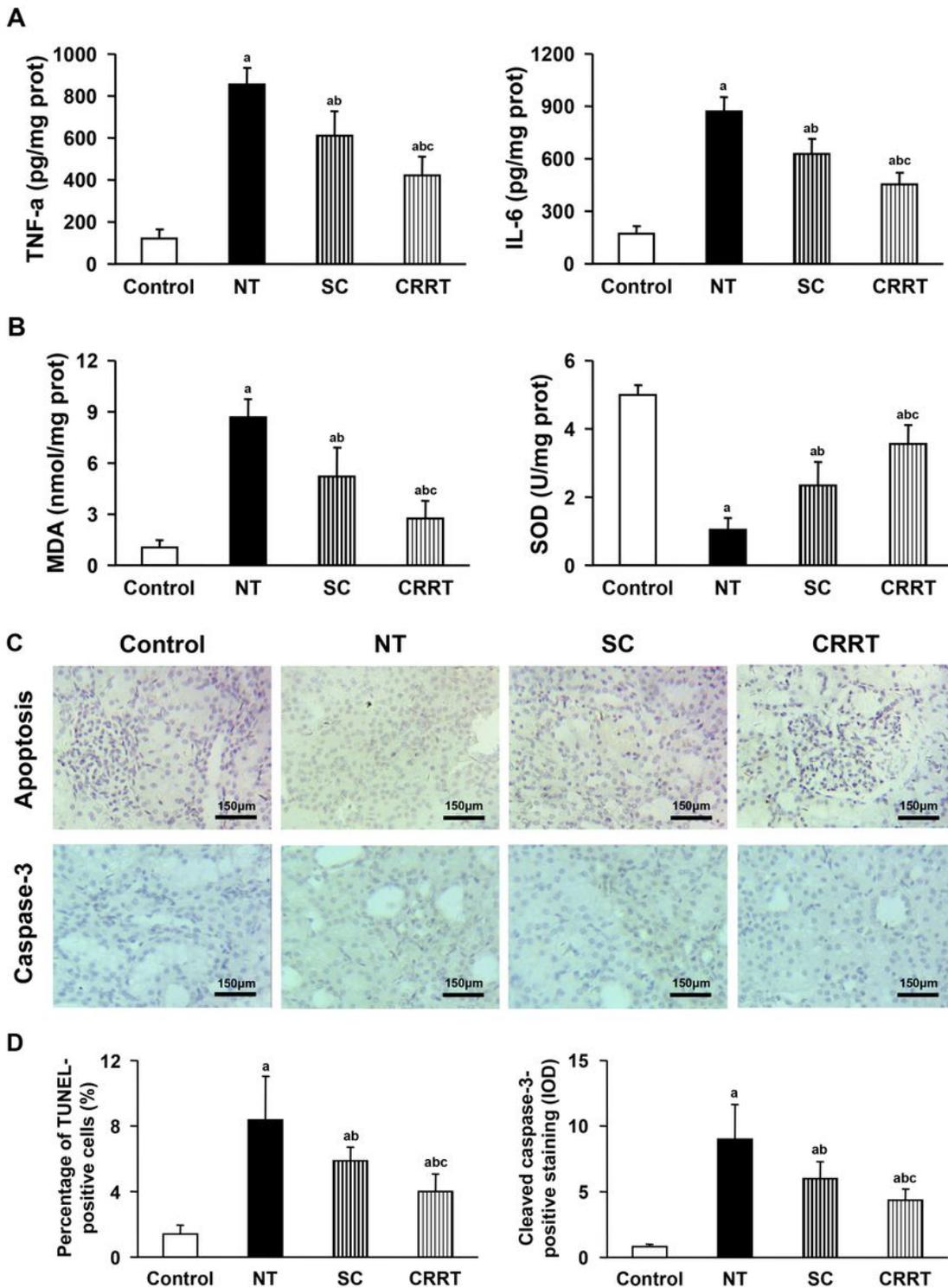


Figure 3

The changes of Cr and BUN in different groups (note: except the Control group containing 5 swine, the other groups have 9 swine each). BL indicates baseline; BUN, blood urea nitrogen; Cr, creatinine; CRRT, continuous renal replacement therapy; DF, defibrillation; NT, normothermia; PC, precordial compression;

SC, surface cooling; VF, ventricular fibrillation. aP< 0.05 versus Control group; bP< 0.05 versus NT group; cP< 0.05 versus SC group.



**Figure 4**

The comparisons of tissue inflammation, oxidative stress and cell apoptosis in kidney in different groups (note: except the Control group containing 5 swine, the other groups have 9 swine each). A, The levels of TNF-α and IL-6; B, The levels of MDA and the activities of SOD; C, Representative

photomicrographs of TUNEL assay and immunostaining of cleaved caspase-3 protein; D, The percentage of TUNEL-positive cells and the IOD values of cleaved caspase-3-positive staining. CRRT indicates continuous renal replacement therapy; IL, interleukin; IOD, integrated optical density; MDA, malondialdehyde; NT, normothermia; SC, surface cooling; SOD, superoxide dismutase; TNF, tumor necrosis factor. aP< 0.05 versus Control group; bP< 0.05 versus NT group; cP< 0.05 versus SC group.

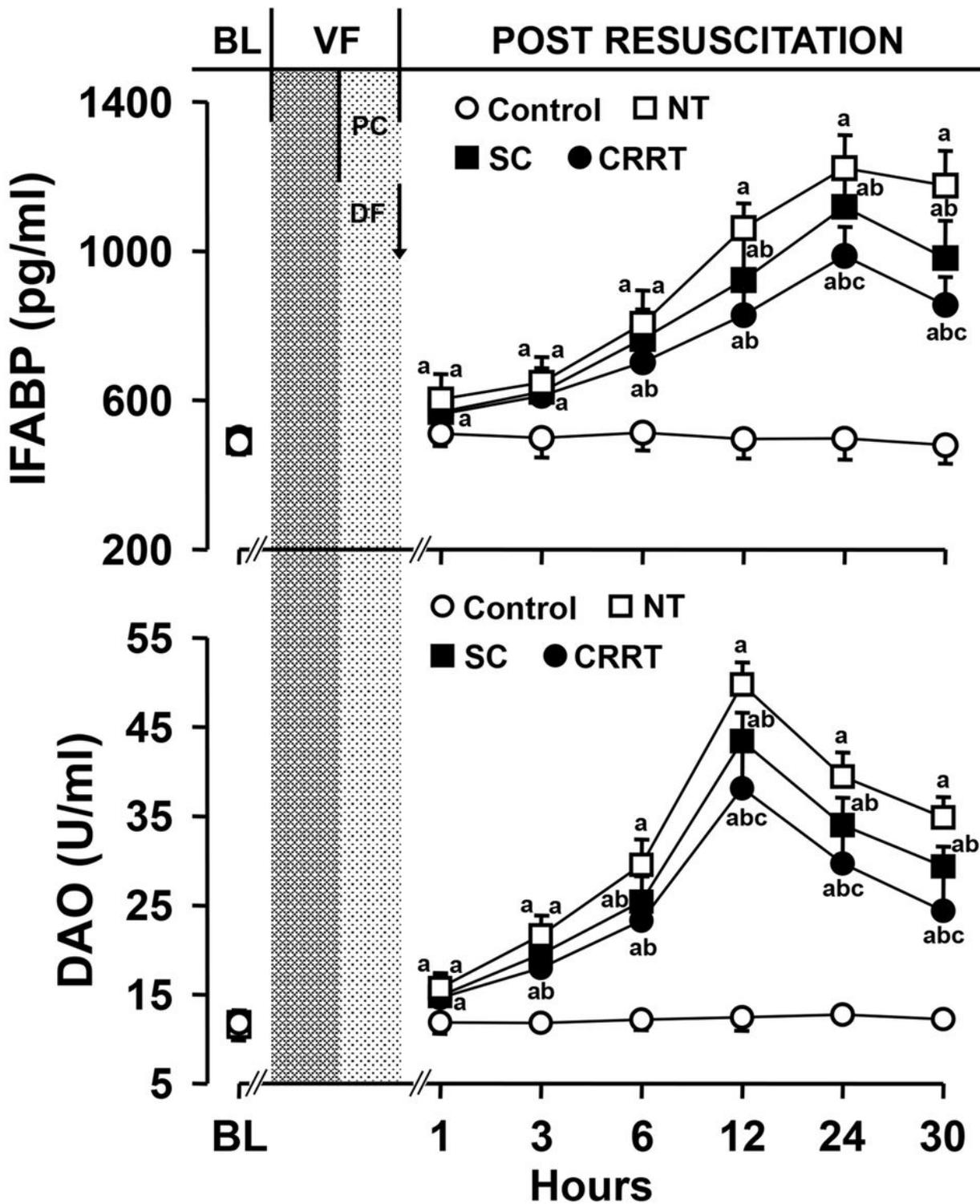


Figure 5

The changes of IFABP and DAO in different groups (note: except the control group containing 5 swine, the other groups have 9 swine each). BL indicates baseline; CRRT, continuous renal replacement therapy; DAO, diamine oxidase; DF, defibrillation; IFABP, intestinal fatty acid binding protein; NT, normothermia; PC, precordial compression; SC, surface cooling; VF, ventricular fibrillation. aP < 0.05 versus control group; bP < 0.05 versus NT group; cP < 0.05 versus SC group.

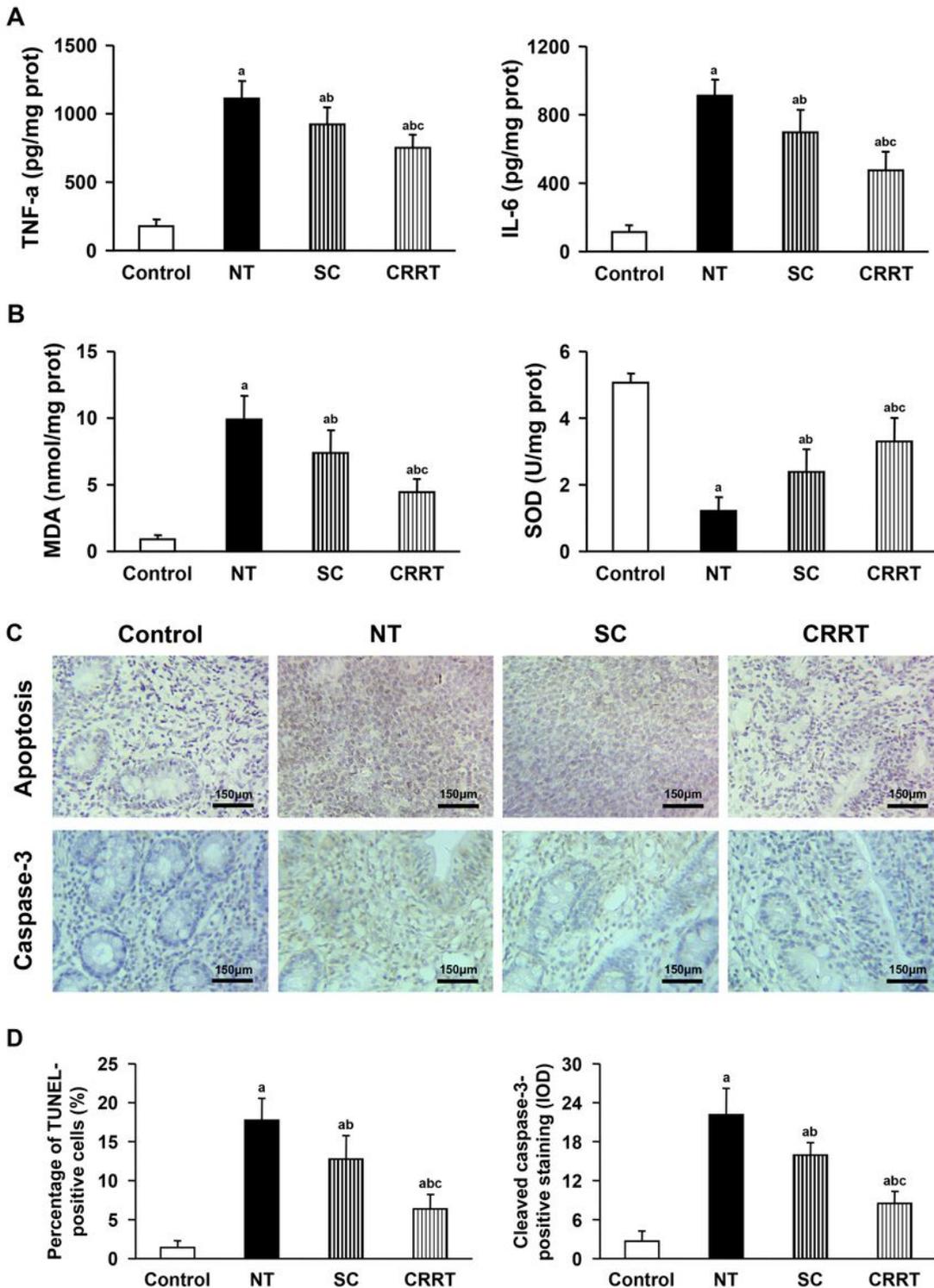


Figure 6

The comparisons of tissue inflammation, oxidative stress and cell apoptosis in intestine in different groups (note: except the control group containing 5 swine, the other groups have 9 swine each). A, The levels of TNF- $\alpha$  and IL-6; B, The levels of MDA and the activities of SOD; C, Representative photomicrographs of TUNEL assay and immunostaining of cleaved caspase-3 protein; D, The percentage of TUNEL-positive cells and the IOD values of cleaved caspase-3-positive staining. CRRT indicates continuous renal replacement therapy; IL, interleukin; IOD, integrated optical density; MDA, malondialdehyde; NT, normothermia; SC, surface cooling; SOD, superoxide dismutase; TNF, tumor necrosis factor. aP < 0.05 versus Control group; bP < 0.05 versus NT group; cP < 0.05 versus SC group.