

# Determination of the Optimal Mean Arterial Pressure for Postbleeding Resuscitation after Hemorrhagic Shock in Rats

Tao Li, Ph.D.,\* Yu Zhu, M.S.,† Yuqiang Fang, Ph.D.,\* Liangming Liu, M.D., Ph.D.‡

## ABSTRACT

**Background:** The authors previously found that 50–60 mmHg mean arterial blood pressure (MAP) was an optimal target resuscitation pressure for hemorrhagic shock before bleeding was controlled in rats. However, the optimal target resuscitation pressure for hemorrhagic shock after bleeding has been controlled has not been determined.

**Methods:** A model of uncontrolled hemorrhagic shock was initiated in anesthetized Wistar rats. After 1-h hypotensive resuscitation and bleeding was stopped, rats received fluid resuscitation to different target MAPs (50, 70, or 90 mmHg) with lactated Ringer's solution (LR), 6% hydroxyethyl starch (HES), LR+HES (2:1) or LR+whole blood (2:1) for 2 h. Animal survival, hemodynamic parameters, and vital organ functions were observed.

**Results:** After bleeding had been controlled, mildly hypotensive resuscitation at a target MAP of 70 mmHg increased the survival time and survival rate compared with a target MAP of 50 mmHg and 90 mmHg ( $P < 0.05$  or  $0.01$ ). Hemodynamic parameters, cardiac output, oxygen delivery, and vital organ function (including mitochondrial function) in 70 mmHg target MAP groups were better than in other

### What We Already Know about This Topic

- Experimental data show that hypotensive fluid resuscitation for uncontrolled hemorrhagic shock may improve success rate of resuscitation before control of bleeding

### What This Article Tells Us That Is New

- In this rat model of uncontrolled hemorrhagic shock, best resuscitation success rates were obtained with mild hypotensive resuscitation maintaining a mean target blood pressure of 70 mmHg after control of bleeding

two-target pressure groups ( $P < 0.05$  or  $0.01$ ). Among the fluids tested, LR+whole blood (2:1) or LR+HES130 (2:1) had better effects than LR or HES alone at each level of target blood pressure.

**Conclusion:** Mildly hypotensive resuscitation is also needed for hemorrhagic shock after bleeding has been controlled, irrespective of whether crystalloids or colloids are used. The optimal target pressure was 70 mmHg in our rat model. A resuscitation pressure that is too low or too high cannot produce a good resuscitative effect.

**P**ERMISSIVE hypotension within volume resuscitative efforts is a “treatment approach” that is sometimes clinically practiced in prehospital scenarios with specific traumatic injury patterns leading to hemorrhage, hypotension, and/or shock. Aggressive fluid resuscitation has traditionally been used after controlled or uncontrolled hemorrhagic shock. Normotensive resuscitation may effectively recover blood pressure, increase tissue perfusion and oxygen delivery, and stabilize hemodynamics.<sup>1–3</sup> Nevertheless, infusion of a large amount of fluid can cause blood loss and severe hemodilution, induce clot dislocation, and reduce the concentration of platelet and coagulant factors, resulting in deterioration of the resuscitation effect (particularly for uncontrolled hemorrhagic shock).<sup>4–5</sup>

In recent years, some experimental data have shown that hypotensive fluid resuscitation may improve resuscitative effects after uncontrolled hemorrhagic shock before bleeding has stopped.<sup>1,6</sup> Our previous study demonstrated that a target mean arterial blood pressure (MAP) of 50–60 mmHg was the optimal hypotensive resuscitation (early resuscitation) pressure for uncontrolled hemorrhagic shock before bleeding was controlled in rats.<sup>7</sup> Apart

\* Associate Professor, State Key Laboratory of Trauma, Burns and Combined Injury, Second Department of Research Institute of Surgery, Daping Hospital, Third Military Medical University, Chongqing, China † Research Assistant, State Key Laboratory of Trauma, Burns and Combined Injury, Second Department of Research Institute of Surgery, Daping Hospital, Third Military Medical University. ‡ Professor, State Key Laboratory of Trauma, Burns and Combined Injury, Second Department of Research Institute of Surgery, Daping Hospital, Third Military Medical University.

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Address correspondence to Dr. Liu: Second Department of Research Institute of Surgery, Daping Hospital, Third Military Medical University, Daping, Chongqing 400042, P. R. China. liangmingliu@yahoo.com. Information on purchasing reprints may be found at [www.anesthesiology.org](http://www.anesthesiology.org) or on the masthead page at the beginning of this issue. ANESTHESIOLOGY's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

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from studies involving experimental animals, there have been some reports about the clinical application of hypotensive resuscitation. The landmark study was by Bickel *et al.*<sup>8</sup> in the 1990s. They advocated delaying fluid resuscitation of hypotensive patients with penetrating injuries to the torso until arrival in the operating theater. They evaluated 598 patients with penetrating injuries to the torso. Among the 289 patients who received delayed fluid resuscitation, 203 (70%) survived and were discharged from hospital, compared with 193 of the 309 patients (62%) who received immediate fluid resuscitation. Hypotensive resuscitation also has been used in the management of combat casualties and trauma patients.<sup>9–11</sup>

After bleeding has been controlled, whether permissive hypotensive resuscitation is also required for hemorrhagic shock is controversial. The current thinking is that resuscitation should be normalized. However, rapid normalization or deliberately providing supernormal resuscitation has not led to good outcomes in the clinic or in the laboratory. For example, Handrigan *et al.*<sup>12</sup> compared the effects of hypotensive resuscitation (MAP = 60 mmHg) with lactated Ringer's solution (LR) Hextend, 6% hydroxyethyl starch in a balanced salt solution, and PolyHeme, a polymerized hemoglobin solution, or standard resuscitation (MAP = 80 mmHg) with LR during hemorrhagic shock with unanesthetized Sprague-Dawley rats. The results showed that there has been a trend toward improved 24-h survival with hypotensive resuscitation.<sup>12</sup> Liu *et al.*<sup>13</sup> found that 100-mmHg resuscitation reduced the hematocrit and vascular reactivity for controlled hemorrhagic shock compared with 70-mmHg resuscitation in rats.

To explore if permissive hypotensive resuscitation is also needed and which pressure and solution are optimal for late resuscitation for hemorrhagic shock after bleeding has been controlled, we compared the resuscitative effects of target MAPs of 50, 70, and 90 mmHg by administering LR, 6% hydroxyethyl starch 130/0.4 (HES130/0.4), LR and HES (2:1), or LR and whole blood (2:1) in a whole-animal rat model.

## Materials and Methods

### Ethical Approval of the Study Protocol

This study was approved by the Research Council and Animal Care and Use Committee of the Research Institute of Surgery, Daping Hospital, Third Military Medical University (Chongqing, China). None of the authors are members of this committee.

### Animal Management

Three hundred sixty male and female Wistar rats (200–250 g) received no food for 12 h, but were allowed water *ad libitum* before the experiment. The number of animals enrolled in the current study was decided by power analyses ( $\alpha$  at 0.05, power at 80%, and with two-tailed analysis). On the day of the experiment, rats were initially

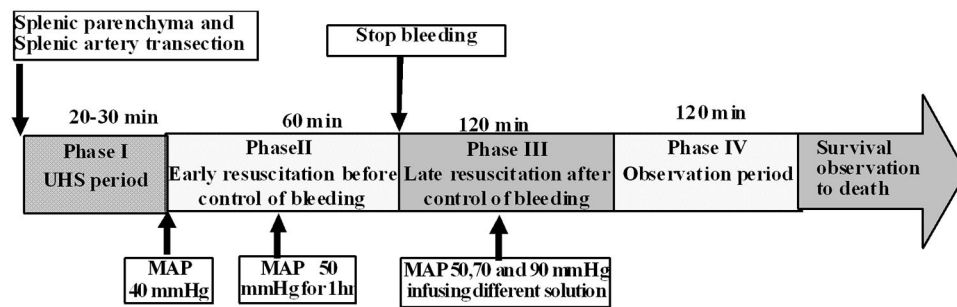
anesthetized with sodium pentobarbital (30 mg/kg, intraperitoneal injection). This was added until there was no response to the needle stimulus; the total amount of sodium pentobarbital was approximately 50 mg/kg. Right femoral arteries and veins were catheterized for monitoring MAP, and for fluid infusion. An uncontrolled hemorrhagic shock model was induced by transection of splenic parenchyma and one of the branches of the splenic artery, as described before in our research team.<sup>14</sup> In general, rats did not need additional sodium pentobarbital during the experiment.

### Experimental Protocol and Phases

Experiments were classified into four phases based on clinical practice. Phase I was the uncontrolled hemorrhagic shock period (“model stage”). In phase I, blood was allowed to freely hemorrhage into the abdominal cavity when splenic parenchyma and one of the branches of the splenic artery were transected. When the MAP decreased to 40 mmHg, uncontrolled hemorrhagic shock was achieved. Phase II was the permissive hypotensive resuscitation period (“early resuscitation period”) before bleeding was controlled. This was in accordance with our previous study<sup>8</sup> in which rats were resuscitated at a target MAP of 50 mmHg for 1 h with infusion of 6% HES130 + LR solution at a ratio of 1:2. Phase III was the definitive resuscitation period (“late resuscitation period”) after bleeding was stopped by full ligation of the splenic artery. Rats received LR solution, 6% HES 130, LR+HES (2:1) or LR+whole blood (2:1) to maintain a target MAP of 50, 70, or 90 mmHg for 2 h, respectively. Normal MAP for Sprague-Dawley rats under approximately 50 mg/kg pentobarbital anesthesia is approximately 90–100 mmHg.<sup>7,15</sup> Whole blood was acquired from donor normal rats. Phase IV was the 2-h observation period (fig. 1). Experiments were undertaken as three series.

### Survival and Hemodynamic Parameters

One hundred twenty rats received left ventricular catheterization in addition to the surgical procedures detailed in the previous paragraphs. After hypotensive resuscitation (phase II), rats were given LR solution, 6% HES130, LR + 6% HES130 (2:1), or LR+whole blood (2:1) to maintain MAP at 50, 70, and 90 mmHg for 2 h (phase III). Rats were further observed for 60 min (phase IV). Each fluid tested at every target MAP involved experiments on 10 rats. Shed blood was not reinfused in all groups. Which group the rats were allocated to was based on the randomization procedure. Blood gases, MAP, left intraventricular systolic pressure, and maximal change rate in left intraventricular pressure ( $\pm dp/dt_{max}$ ) were determined by a polygraph physiologic recorder (SP844, Power Laboratory; AD Instruments, Castle Hill, NSW, Australia) and blood gas analyzer (Phox plus L, Nova Biomedical, Waltham, MA) at baseline, at the end of



**Fig. 1.** Timeline of the experimental phases. MAP = mean arterial blood pressure; UHS = uncontrolled hemorrhagic shock.

phase I and phase II, at 1 h and 2 h during phase III, and at the end of phase IV. After the acute observation period, catheters were removed and incisions closed. Rats were returned to the Animal Observation Center to record the survival time.

### Cardiac Output, Oxygen Delivery ( $DO_2$ ), and Oxygen Consumption ( $VO_2$ )

In this experiment, another 120 rats received catheterization of the left ventricle and right external jugular vein for measurement of cardiac output. The other procedures (including grouping, production of hemorrhage shock, and fluid infusion) were the same as described in experiment series 1. Each fluid tested at every target MAP involved experiments on 10 rats. Two hours after fluid infusion during phase III, the cardiac output was measured by a Cardiomax-III machine (Columbus Instruments, Columbus, OH). Blood was sampled from the femoral artery and femoral vein to analyze arterial and venous blood gases, respectively, using a blood gas analyzer (Phox plus L). The values of  $DO_2$  and  $VO_2$  in tissue were calculated using the following equations:

$$DO_2 = CI \times 13.4 \times \text{hemoglobin} \times SaO_2$$

$$VO_2 = CI \times 13.4 \times \text{hemoglobin} \times (SaO_2 - SvO_2)$$

### Water Content and Tissue Blood Flow of Vital Organs as Well as Their Function

In this experiment, rats underwent laparotomy but without left ventricular catheterization. The procedures for grouping, induction of hemorrhage shock, and fluid infusion were as described previously. Each group had 10 rats. The total number of rats 6 series was also 120. After fluid infusion for 2 h to maintain the target MAP at 50, 70, and 90 mmHg (phase III), blood flow in the liver and kidney was measured by a Laser Doppler Blood Flowmeter (Periflux system 5000, Primed, Stockholm, Sweden). Blood was sampled from a catheter on the femoral vein to measure the concentration of alanine aminotransferase, aspartate aminotransferase, creatinine, and blood urea nitrogen using a chemical analyzer (LX20, Beckman Coulter, Brea, CA). Rats were then killed to obtain the liver, kidneys, and

lungs to measure the water content (presented as the ratio of dry/wet weight) and mitochondrial function. The latter was reflected by the respiratory control rate (the ratio of the rate of consumed oxygen with and without adenosine diphosphate). The measurement method was as described in our previous study.<sup>7</sup>

### Statistical Analyses

Data (MAP, left ventricular pressure,  $\pm dp/dt$  max, oxygen pressure, blood pH value, cardiac output,  $DO_2$ ,  $VO_2$ , fluid requirement, blood loss, hematocrit, mitochondrial respiratory control rate, and organ function) are the mean  $\pm$  SD of *n* observations. Statistical differences were analyzed by a repeated measures one-way or two-way ANOVA (different fluids at different target resuscitation pressure *vs.* time points), followed by the *post hoc* Tukey test (SPSS 15.0, SPSS Incorporated, Chicago, IL). Before the application of ANOVA, the distribution of data was assessed using the Kolmogorov–Smirnov test. The results showed that all parametric data were normally distributed. Survival time and survival prevalence were analyzed by median and interquartile range, Kaplan–Meier survival analyses, and the log-rank test.  $P < 0.05$  was considered significant (two-tailed).

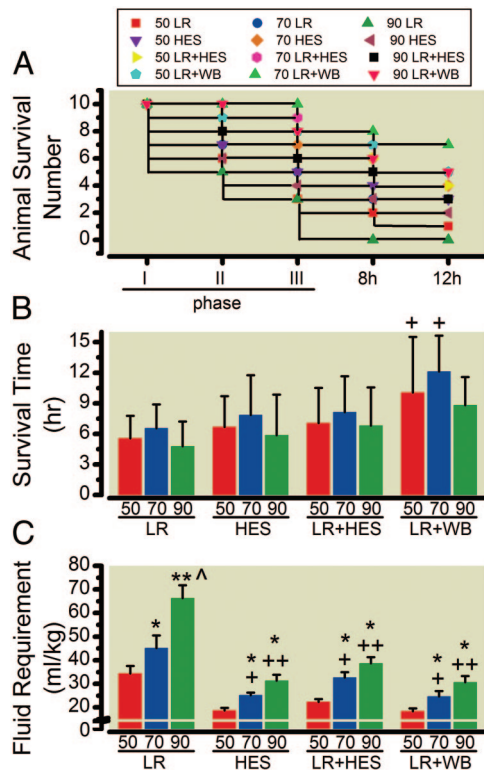
## Results

### Animal Survival, Hemodynamics, and Blood Gases

**Animal Survival.** Among the different target resuscitation pressures for each fluid, the 70 mmHg pressure group had a better 12-h survival value (30%, 40%, 50%, and 70% in LR, HES, LR + 6% HES130, or LR+whole blood group) than the 50 mmHg (10%, 30%, 40%, and 50%) and 90 mmHg groups (0, 20%, 30%, and 50%). Animal survival time in the 70 mmHg pressure group for each fluid was longer than that in the 50 mmHg and 90 mmHg groups. Among all fluids, LR+whole blood at a resuscitation pressure of 70 mmHg had the best 12-h survival value (70%) and survival time (fig. 2, A and B).

**Fluid Requirement.** The volume requirement of LR, 6% HES130 or LR + 6% HES130, and LR+whole blood to maintain a target MAP at 50, 70, or 90 mmHg for 2 h was significantly different. The required volume for LR to maintain an identical MAP was threefold to fourfold more than





**Fig. 2.** Survival and fluid requirements. (A) 12-h survival rate. (B) Survival time. (C) Fluid requirement. Survival time and survival rate were represented by median and Kaplan–Meier survival line and analyzed by interquartile range and Kaplan–Meier survival analyses, respectively. Fluid requirement is represented as mean  $\pm$  SD ( $n = 10$ ). HES = hydroxyethyl starch; LR = lactated Ringer’s solution; MAP = mean arterial blood pressure; WB = whole blood. 50, 70, and 90 in x-axis means maintaining MAP at 50, 70, and 90 mmHg with LR, HES, or LR+HES and LR+whole blood. \* $P < 0.05$ , \*\* $P < 0.01$  versus 50 mmHg group in the same fluid groups; ^  $P < 0.05$  versus 70 mmHg group in the same fluid groups; +  $P < 0.05$ , ++  $P < 0.01$  versus the same MAP level of the LR group.

the 6% HES130, LR + 6% HES130 or LR+whole blood group. The LR+whole blood group needed the smallest volume among all the fluids tested (fig. 2C).

**MAP.** LR, 6% HES130, LR + 6% HES130 (2:1), or LR+whole blood (2:1) could maintain a target MAP of 50, 70, or 90 mmHg for 2 h (phase III). After fluid infusion at phase III, the MAP in the 90 mmHg target pressure group for all solutions was decreased, whereas the MAP in the 6% HES130, LR + 6% HES130, or LR+whole blood groups at 50 and 70 mmHg target pressure was increased ( $P < 0.01$ ). The 70 mmHg target pressure group in all solutions had a larger increase in MAP than the other two pressures groups (fig. 3A).

**Left Ventricular Systolic Pressure and  $\pm$ dp/dt max.** Left ventricular systolic pressure and  $\pm$ dp/dt max were decreased after hemorrhagic shock, and increased during the period of fluid infusion (phase III). Among different target resuscitation pressures in the same fluid, the 70 mmHg target pressure

group had a higher left ventricular systolic pressure and  $\pm$ dp/dt max than the 50 and 90 mmHg groups. Among different fluids, LR + 6% HES130 (2:1) or the LR+whole blood group improved left ventricular systolic pressure and  $\pm$ dp/dt max more than the LR or HES groups (fig. 3, B, C, and D).

**Blood Gases.** At the end of phase II, some acidosis was noted in all groups. The pH value was decreased compared with baseline (data not shown in table 1). All fluids with a target pressure resuscitation of 50 mmHg did not improve the pH value, whereas target pressure resuscitations of 70 and 90 mmHg did. There were no significant differences among different solutions. For the oxygen pressure in the artery, during and after fluid infusion, LR+whole blood increased the oxygen pressure (which was higher than that for the other fluids). There were no significant differences among LR, HES and LR+HES groups (table 1).

### Cardiac Output and $DO_2$ and $VO_2$ of Tissue

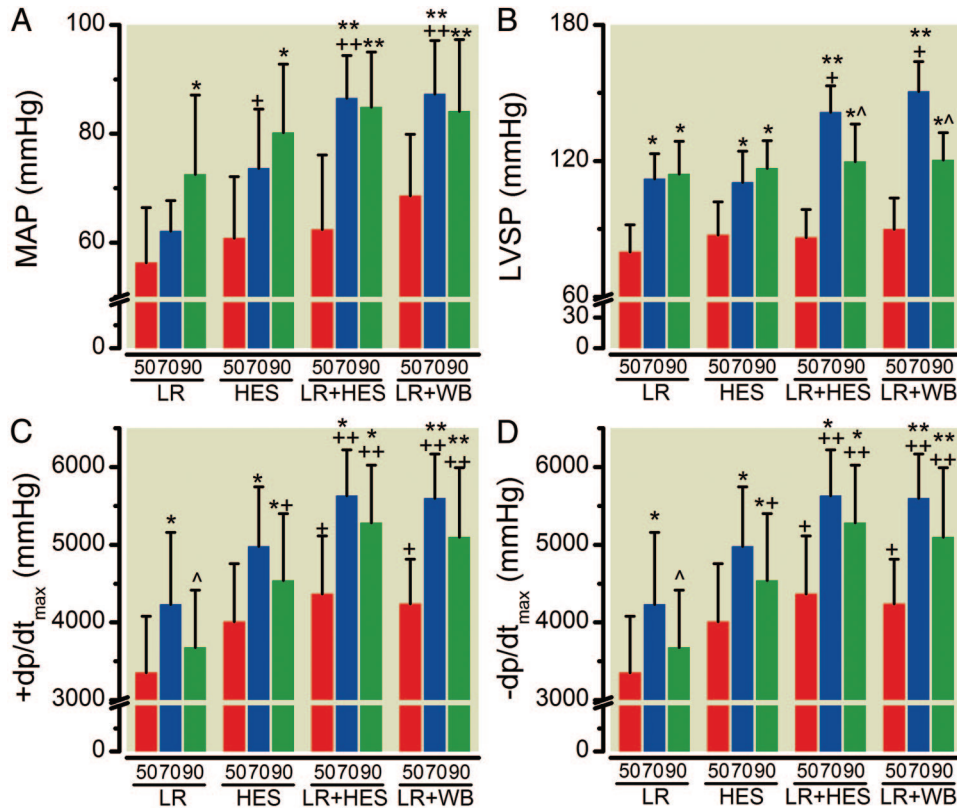
**Cardiac Output.** Cardiac output was decreased after hemorrhagic shock, and increased during the period of fluid infusion (phase III). Among different target resuscitation pressures in the same fluid, target resuscitation pressures of 70 and 90 mmHg increased the cardiac output compared with a resuscitation pressure of 50 mmHg ( $P < 0.05$ ). There were no significant differences in the 70 and 90 mmHg target resuscitation pressure groups and in the LR + 6% HES130 and LR+whole blood groups (fig. 4, A).

**$DO_2$  and  $VO_2$ .**  $DO_2$  and  $VO_2$  were decreased after hemorrhagic shock, and increased after fluid infusion (phase III). Among different target resuscitation pressures in the same fluid, target resuscitation pressures of 70 and 90 mmHg increased  $DO_2$  and  $VO_2$  higher than that seen in the 50 mmHg resuscitation pressure group ( $P < 0.05$  or  $P < 0.01$ ). The LR + 6% HES130 and LR+whole blood groups were superior to LR solution or HES used alone. There was no significant difference between the 70 and 90 mmHg target resuscitation pressure groups (fig. 4, B and C).

### Tissue Blood Flow, Organ Function, and Mitochondrial Function in the Liver and Kidney

**Tissue Blood Flow in the Liver and Kidney.** Tissue blood flow in the liver and kidney was decreased after hemorrhagic shock. Fluid infusion increased blood flow in these organs. The LR + 6% HES130 or LR+whole blood groups at 70 mmHg target pressure had better blood flow in the liver (135 U/min and 199 U/min) and kidney (146 U/min and 224 U/min) than other fluids and at other target pressures. LR+whole blood at 70 mmHg had the best effect (fig. 5, A and B).

**Mitochondrial Function in the Liver and Kidney.** The respiratory control rate of mitochondria in the liver and kidney was decreased after shock. Fluid resuscitation, irrespective of whether it was with LR, 6% HES, LR + 6% HES, or LR+whole blood, could improve the respiratory control rate



**Fig. 3.** MAP, LVSP, and  $\pm dp/dt_{max}$  at the end of 2-h fluid infusion (phase III). (A) MAP. (B) LVSP. (C)  $+dp/dt_{max}$ . (D)  $-dp/dt_{max}$ . Data are expressed as mean  $\pm$  SD (n = 10). HES = hydroxyethyl starch. LR = lactated Ringer’s solution; LVSP = left intraventricular systolic pressure; MAP = mean arterial blood pressure;  $\pm dp/dt_{max}$  = maximal change rate in left intraventricular pressure; WB = whole blood. 50, 70, 90 in x-axis means maintaining MAP at 50, 70, and 90 mmHg with LR, HES, or LR+HES and LR+whole blood. \*  $P < 0.05$ , \*\*  $P < 0.01$  versus 50 mmHg group in the same fluid groups;  $^{\wedge} P < 0.05$  versus 70 mmHg group in the same fluid groups; +  $P < 0.05$ , ++  $P < 0.01$  versus the same MAP level of the LR group.

of mitochondria. The LR + 6% HES130 and LR+whole blood groups at 70 mmHg target pressure improved the respiratory control rate of mitochondria in the liver and kidney better than the other fluid groups and at other target

pressures. LR+whole blood at 70 mmHg had the best effect (fig. 5, C and D).

**Liver and Kidney Function.** After hemorrhagic shock, the variables reflecting liver and kidney function and hepatic cell

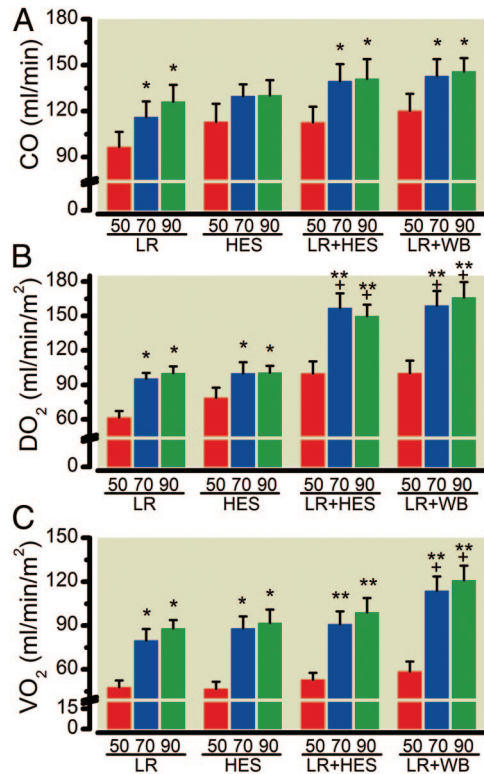
**Table 1.** Blood pH and  $Pao_2$

Group	pH			$Pao_2$ (mmHg)		
	Phase II	Phase III	Phase IV	Phase II	Phase III	Phase IV
LR						
50	7.31 $\pm$ 0.03	7.26 $\pm$ 0.08	7.29 $\pm$ 0.06	129.3 $\pm$ 17.3	110.4 $\pm$ 9.6	107.8 $\pm$ 10.1
70	7.37 $\pm$ 0.07	7.27 $\pm$ 0.08	7.31 $\pm$ 0.08	137.5 $\pm$ 23.4	111.6 $\pm$ 26.7	118.2 $\pm$ 18.7*
90	7.34 $\pm$ 0.05	7.38 $\pm$ 0.0*	7.41 $\pm$ 0.02**	133.6 $\pm$ 13.9	96.2 $\pm$ 19.8	90.3 $\pm$ 19.8
HES						
50	7.32 $\pm$ 0.06	7.27 $\pm$ 0.02	7.28 $\pm$ 0.06	119.1 $\pm$ 16.7	112.3 $\pm$ 16.7	104.5 $\pm$ 15.6
70	7.32 $\pm$ 0.07	7.34 $\pm$ 0.03*	7.35 $\pm$ 0.01*	126.1 $\pm$ 16.9	107.4 $\pm$ 14.5	111.3 $\pm$ 15.9
90	7.29 $\pm$ 0.07	7.39 $\pm$ 0.02*	7.41 $\pm$ 0.02**	126.4 $\pm$ 17.4	101.4 $\pm$ 19.2	102.1 $\pm$ 18.7
LR+HES						
50	7.31 $\pm$ 0.04	7.28 $\pm$ 0.06	7.29 $\pm$ 0.08	129.1 $\pm$ 19.8	110.1 $\pm$ 13.4	105.2 $\pm$ 21.2
70	7.35 $\pm$ 0.03	7.32 $\pm$ 0.01	7.34 $\pm$ 0.01	127.7 $\pm$ 9.0	107.8 $\pm$ 10.8	114.6 $\pm$ 14.6
90	7.35 $\pm$ 0.02	7.33 $\pm$ 0.09*	7.39 $\pm$ 0.06*	125.5 $\pm$ 18.7	107.3 $\pm$ 16.9	106.5 $\pm$ 17.5
Whole blood						
50	7.32 $\pm$ 0.02	7.27 $\pm$ 0.06	7.27 $\pm$ 0.08	128.9 $\pm$ 18.1	139.3 $\pm$ 15.3+	115.2 $\pm$ 19.9
70	7.33 $\pm$ 0.03	7.33 $\pm$ 0.04	7.34 $\pm$ 0.03	126.5 $\pm$ 10.8	147.9 $\pm$ 10.4++	134.5 $\pm$ 15.4+
90	7.30 $\pm$ 0.09	7.35 $\pm$ 0.09*	7.39 $\pm$ 0.03*	124.6 $\pm$ 12.1	155.6 $\pm$ 11.5*++	126.4 $\pm$ 16.9++

50, 70, 90 means MAP was maintained at 50, 70, 90 mmHg with LR, HES, or LR+HES and LR+whole blood.

\*  $P < 0.05$ , \*\*  $P < 0.01$  vs. 50 mmHg group in the same fluid; +  $P < 0.05$ , ++  $P < 0.01$  vs. LR group at the same target MAP.

HES = hydroxyethyl starch; LR = lactated Ringer’s solution; MAP = mean arterial pressure;  $Pao_2$  = arterial oxygen pressure; Phase II = early resuscitation period before bleeding was controlled; Phase III = late resuscitation after bleeding had been controlled; Phase IV = observation period.



**Fig. 4.** Cardiac output (CO, A), oxygen delivery (DO<sub>2</sub>, B) and oxygen consumption (VO<sub>2</sub>, C) at the end of 2 h fluid infusion (phase III). Data are expressed as mean  $\pm$  SD (n = 10); HES = hydroxyethyl starch; LR = lactated Ringer's solution; MAP = mean arterial blood pressure; WB = whole blood. 50, 70, and 90 in x-axis means maintaining MAP at 50, 70, and 90 mmHg with LR, HES, or LR+HES and LR+whole blood. \*  $P < 0.05$ . \*\*  $P < 0.01$  versus 50 mmHg group in the same fluid groups. +  $P < 0.05$  versus the same MAP level of the LR group.

damage indices (including levels of alanine aminotransferase, aspartate aminotransferase, creatinine, and blood urea nitrogen) were increased. A target resuscitation pressure of 50 mmHg did not improve these variables. A target resuscitation pressure of 70 mmHg improved these variables, whereas a target resuscitation pressure of 90 mmHg had some harmful effects on these variables. LR + 6% HES130 and LR+whole blood at a target resuscitation pressure of 70 mmHg were better than other fluids and at other target pressures (figs. 6A–D).

**Water Content in the Lung, Liver, and Kidney.** Fluid resuscitation at a target pressure of 50 mmHg did not cause an obvious increase in water content in the lung, liver, and kidney. There was no significant difference in the dry/wet weight ratio in these organs among all the fluids tested. Infusion with LR solution to a target pressure of 70 or 90 mmHg for 2 h caused a significant increase in the water content in these organs. Infusion with 6% HES130, LR + 6% HES130, or whole blood to a target pressure of 70 mmHg for 2 h had no significant influence on the water content in these organs. However, infusion to a target

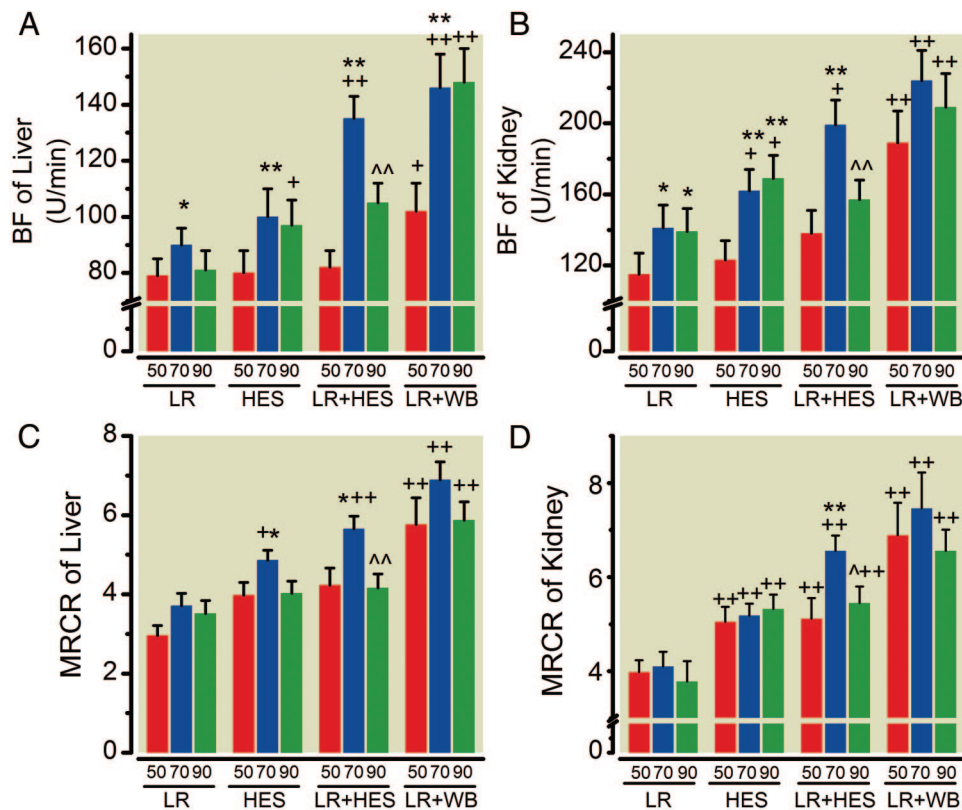
pressure of 90 mmHg for 2 h with these fluids caused an increase in water content in these organs (fig. 7).

## Discussion

Using an uncontrolled hemorrhagic shock rat model, we compared the resuscitative effects of infusion of LR solution, 6% HES130, LR + 6% HES130 (2:1), or LR+whole blood to a target pressure of 50 (underresuscitation), 70 (mildly hypotensive resuscitation), and 90 (normotensive- resuscitation) mmHg after bleeding had stopped. The results showed that resuscitation at a target pressure of 50 mmHg, irrespective of whatever solution was used, did not maintain hemodynamic stability and did not maintain sufficient perfusion of tissue. A target resuscitation pressure maintained at 70 mmHg with LR solution, 6% HES130, LR + 6% HES130, or whole blood could better maintain hemodynamic stability and prolong the survival time than a target resuscitation pressure maintained at 50 and 90 mmHg. LR + 6% HES130 and whole blood had a better effect than LR solution and HES alone. LR+whole blood at 70 mmHg had the best effect. Resuscitation at a target pressure of 90 mmHg did not further improve the resuscitative effects, but instead increased the water content of the lungs, liver, and kidneys and decreased tissue perfusion and organ functions. Among these solutions, LR solution at a resuscitation pressure of 90 mmHg had the worst results: it caused the most obvious tissue edema, the worst function in the liver and kidneys, and was associated with the lowest animal survival. These results suggested that hemorrhagic shock after bleeding had been controlled also merited permissive hypotensive resuscitation for a certain time period in our rat model. Pressure that is too low or too high cannot produce a good resuscitative effect after bleeding has been controlled. The optimal target resuscitative pressure was found to be 70 mmHg irrespective of whether crystalloid or colloid solution was used. Whole blood also required permissive target hypotension.

The current study indicated that LR+HES130 or LR+whole blood infused at a ratio of 2:1 at a target pressure of 70 mmHg elicited the best resuscitation results. Apart from improving hemodynamic parameters (including left intraventricular systolic pressure,  $\pm$  dp/dt max and cardiac output), these two resuscitation regimens also improved the tissue blood flow, DO<sub>2</sub>, VO<sub>2</sub>, and vital organ functions (including mitochondrial function). The main reasons are that different fluids have different physical and chemical properties, and different target pressures result in different levels of tissue perfusion (which cause different tissue oxygen delivery) and different tissue damage. Whole blood has good oxygen-carrying function, and HES has good volume expansion efficiency (6% HES has 150–200% volume expansion)<sup>16</sup> and microcirculation improvement.<sup>17</sup> Hence, whole blood or HES in combination with LR at an appropriate resuscitation pressure (70 mmHg) improved vital organ





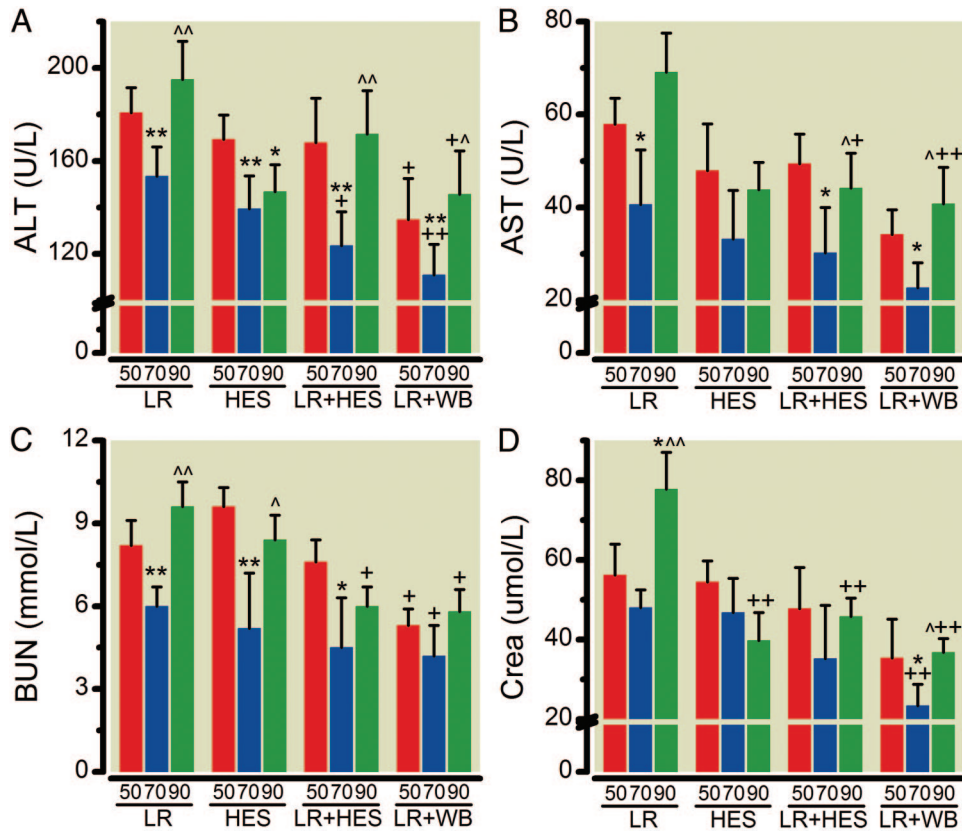
**Fig. 5.** Blood flow and mitochondrial function in the liver and kidney at the end of 2 h fluid infusion (phase III). (A) Liver blood flow. (B) Kidney blood flow. (C) Respiration control rate of liver mitochondria. (D) Respiration control rate of kidney mitochondria. Data are expressed as mean  $\pm$  SD ( $n = 10$ ). BF = blood flow; HES = hydroxyethyl starch; LR = lactated Ringer's solution; MAP = mean arterial blood pressure; MRCR = respiration control rate of mitochondria; WB = whole blood. 50, 70, and 90 in x-axis means maintaining MAP at 50, 70, and 90 mmHg with LR, HES, or LR+HES and LR+whole blood. \*  $P < 0.05$ , \*\*  $P < 0.01$  versus 50 mmHg group in the same fluid groups. ^  $P < 0.05$ , ^^  $P < 0.01$  versus 70 mmHg group in the same fluid groups. +  $P < 0.05$ , ++  $P < 0.01$  versus the same MAP level of the LR group.

function (including mitochondrial function) and animal survival in our rat model.

Hemorrhagic coagulopathy is a significant complication after traumatic injury, and has an important effect on the development and therapy of traumatic hemorrhagic shock. Severe injury or the late stages of trauma can cause hypocoagulable blood.<sup>18–20</sup> The literature has shown that many treatment approaches (including fluid resuscitation) can interfere with coagulation. LR itself did not alter the clotting process. However, a large amount of LR infusion could worsen the existing consumptive coagulopathy because of hemodilution.<sup>21</sup> Whole blood is often used to correct severe trauma or hemorrhage-induced coagulopathy.<sup>22</sup> The synthetic plasma expander HES can compromise blood coagulation. The mechanisms underlying this process include HES decreasing the expression of the platelet surface GPIIb–IIIa receptor and impairing the adhesion and aggregation of platelets. HES can also interfere with the polymerization of fibrin monomers.<sup>23</sup> Any of these effects may have significantly contributed to the results of our study, although coagulatory parameters have not been analyzed.

In addition, we also found that, irrespective of which solution was used, a target resuscitation pressure that was too low or too high could not produce good resuscitative effects for hemorrhagic shock after bleeding had been controlled: only mildly hypotensive resuscitation could produce good resuscitative effects. The reason is that too low a target pressure cannot meet the basic demand of tissue perfusion. Alternatively, too high a target pressure resulted in severe tissue edema and further hemodilution. Mildly hypotensive resuscitation not only met the demand of tissue perfusion, but also did not induce the tissue edema and organ damage in our rat model. The precise mechanism why mildly hypotensive resuscitation can produce good resuscitative effects needs further investigation.

There are many models to mimic uncontrolled hemorrhagic shock.<sup>24–25</sup> Organ damage (including impairment of the liver or spleen in combination with vascular injury) can better imitate clinical uncontrolled hemorrhagic shock.<sup>26</sup> Pure vascular injury (*e.g.*, tail transection) can also result in uncontrolled hemorrhagic shock, coagulation disorders, and inflammatory reactions.<sup>27–29</sup> However, this model cannot completely mimic the scenario of uncontrolled hemorrhagic



**Fig. 6.** Liver and kidney function at the end of 2-h fluid infusion (phase III). (A) ALT. (B) AST. (C) BUN. (D) Crea. Data are expressed as mean  $\pm$  SD ( $n = 10$ ). ALT = alanine aminotransferase; AST = aspartate aminotransferase; BUN = blood urea nitrogen; Crea = serum creatinine; HES = hydroxyethyl starch; LR = lactated Ringer's solution; MAP = mean arterial blood pressure; WB = whole blood. 50, 70, and 90 in x-axis means maintaining MAP at 50, 70, and 90 mmHg with LR, HES, or LR+HES and LR+whole blood. \*  $P < 0.05$ . \*\*  $P < 0.01$  versus 50 mmHg group in the same fluid groups. ^  $P < 0.05$ . ~  $P < 0.01$  versus 70 mmHg group in the same fluid groups. +  $P < 0.05$ , ++  $P < 0.01$  versus the same MAP level of the LR group.

shock because there is almost no tissue damage. Hence, damage to splenic parenchyma and the splenic artery transection model was adopted in the current study to better mimic the clinical situation of uncontrolled hemorrhagic shock. This model was stable and reproducible. We previously used this model to investigate the effect of initial fluid resuscitation on uncontrolled hemorrhagic shock and achieved satisfactory results.<sup>30</sup>

Our previous study showed that a target pressure of 50–60 mmHg in the early resuscitation period was the optimal resuscitation pressure to obtain a beneficial effect for uncontrolled hemorrhagic shock.<sup>7</sup> Sixty minutes of hypotensive resuscitation elicited the best results. More than 90 min of hypotensive resuscitation would affect subsequent resuscitation. Hence, 60 min of hypotensive resuscitation at 50 mmHg was adopted in phase II (early resuscitation period) in the current study. Our previous study showed that the time to reach full resuscitation for hemorrhagic shock in rats was approximately 2–3 h, so a 2-h late resuscitation period (phase III) was selected in the current study.<sup>7</sup> The main objective of using this phase was to observe the effect of mildly hypotensive resuscitation (70 mmHg) on hemorrhagic shock after bleeding had

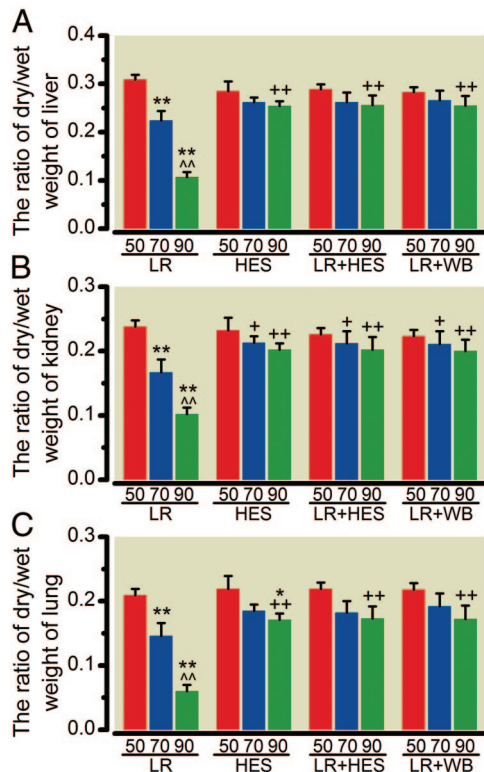
been controlled. After this period of resuscitation, normotension should be attempted. However, to observe the effect of mildly hypotensive resuscitation on subsequent results, a 2-h observation period was selected. Investigation of the effect of normotension after mildly hypotensive resuscitation on the overall results of resuscitation needs further investigation.

The current study had limitations. First, we did not observe the effects of different resuscitation fluids on the coagulation system. Second, we did not observe the effect of normotension after mild hypotensive resuscitation on the overall results. Third, we used a rat model; whether these results can be extrapolated to larger animals or humans requires confirmation. Fourth, only a few solutions were compared in the current study: whether permissive target hypotension is identical for other solutions needs further investigation.

## Conclusion

LR+ 6% HES130 or LR+whole blood at a ratio of 2:1 infused at 70 mmHg of mildly hypotensive resuscitation elicited the best resuscitation results. Mildly hypotensive resus-





**Fig. 7.** Change in the ratio of dry/wet weight of the lungs, liver and kidneys at the end of 2 h fluid infusion (phase III). (A) Lung. (B) Liver. (C) Kidney. HES = hydroxyethyl starch; LR = lactated Ringer's solution; WB = whole blood. Data are expressed as mean  $\pm$  SD ( $n = 10$ ). 50, 70, 90 in x-axis means maintaining MAP at 50, 70, and 90 mmHg with LR, HES, or LR+HES and LR+whole blood. \*  $P < 0.05$ . \*\*  $P < 0.01$  versus 50 mmHg group in the same fluid groups. ^  $P < 0.01$  versus 70 mmHg group in the same fluid group. +  $P < 0.05$ , ++  $P < 0.01$  versus the same MAP level of the LR group.

citation is also needed for hemorrhagic shock after the control of bleeding irrespective of whether crystalloids or colloids are used. The optimal target pressure was 70 mmHg. A resuscitation pressure that is too low or too high cannot produce a good resuscitative effect. Even whole blood could provide the best achievable resuscitation only if mean blood pressures were maintained at a mildly hypotensive level (approximately 70 mmHg) after bleeding had ceased in our rat model.

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