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ORIGINAL ARTICLE

# Effects of crystalloids and colloids on microcirculation, central venous oxygen saturation, and central venous-to-arterial carbon dioxide gap in a rabbit model of hemorrhagic shock

**Makiko Komori[1] · Yuriko Samejima[1] · Keiko Okamura[1] · Junko Ichikawa[1] · Mitsuharu Kodaka[1] · Keiko Nishiyama[1] · Yasuko Tomizawa[2]**

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## Abstract

**Objective** The effects of hydroxyethyl starch (HES) on microcirculation, central venous oxygen saturation ($ScvO_2$), and the central venous-to-arterial carbon dioxide gap ($dCO_2$) are studied in a rabbit model of hemorrhagic shock for elucidating the advantages and drawbacks of resuscitation with HES compared with crystalloids.

**Methods** An ear chamber and sublingual mucosa were used to examine blood vessels by intravital microscopy. Hemorrhagic shock was induced by removing nearly half of the blood volume. Twenty-two rabbits received 20 mL of HES by intravenous infusion immediately after bloodletting. Additional HES was then administered intravenously to a total volume of 100 mL. The other 22 rabbits (control) were intravenously given 40 mL of normal saline solution (NSS), followed by additional NSS to a total volume of 200 mL, administered under the same conditions as HES.

**Results** After the infusion, the vessel density and perfusion rate of the sublingual microcirculation recovered in the HES group. The arteriolar diameter, blood flow velocity, and blood flow rate of the ear microcirculation were maintained in this group, and microcirculatory failure did not develop. In the NSS group, however, all 5 of the aforementioned measured variables were significantly smaller than those in the HES group after the completion of infusion. The recovery of $ScvO_2$ and $dCO_2$ to the respective baseline values was significantly better in the HES group than in the NSS group.

**Conclusion** Intravenous infusion of HES effectively maintains adequate tissue oxygenation and perfusion in hemorrhagic shock.

**Keywords** Rabbit ear chamber · Sublingual · Microvascular change · Hydroxyethyl starch · Fluid resuscitation

## Introduction

The main therapeutic goals of fluid resuscitation in hypovolemia are not only to reestablish macrohemodynamic function, but also to restore the microcirculation and tissue oxygenation. Rapid restitution of intravascular volume is essential to maintain blood flow and oxygen delivery to vital organs. Colloid and crystalloid solutions are two major intravascular replacement regimens for restoring hemodynamic stability after massive hemorrhage [1, 2]. Colloid solutions have been shown to be superior to crystalloid solutions for fluid resuscitation [3–6]. However, which solution is safer in patients during fluid resuscitation remains controversial. The safe use of even a third-generation hydroxyethyl starch (HES) 130/0.4, which has been clinically studied in detail, is supported by minimal evidence [7]. It is thus important to understand the advantages and drawbacks of resuscitation with HES as compared with crystalloids.

Central venous oxygen saturation ($ScvO_2$) is a good substitute for mixed venous oxygen saturation and a sensitive indicator of the balance between oxygen supply and demand [8]. $ScvO_2$ highly correlates with oxygen extraction. During the perioperative period, a low postoperative $ScvO_2$ was associated with increased complications in high-risk surgical patients [9]. The Surviving Sepsis

✉ Makiko Komori
komorim@nifty.com

[1] Department of Anesthesiology, Medical Center East, Tokyo Women's Medical University, 2-1-10, Nishiogu, Arakawa-ku, Tokyo 116-8567, Japan

[2] Department of Cardiovascular Surgery, Tokyo Women's Medical University, 8-1, Kawadacho Shinjuku-ku, Tokyo 162-8666, Japan

Campaign [10] recommends that the treatment of septic shock should be guided by $ScvO_2$, the goal being to elevate it above 70%. However, some studies of septic models showed [11] that $ScvO_2$ was elevated while tissue hypoxia was still present in a majority of patients with septic shock who had already been resuscitated. Thus, $ScvO_2$ does not always reflect tissue hypoxia and might not be the best trigger for starting hemodynamic resuscitation [12]. Correlations between $ScvO_2$ and microcirculation thus have to be studied in a model other than the septic one.

Recently, it was reported [13] that the central venous-to-arterial carbon dioxide gap ($dCO_2$) is a useful index of hypoxemia-induced anaerobic metabolism in hemorrhagic shock. In anaerobic metabolism, hydrogen ions are generated by hydrolysis of adenosine triphosphate to adenosine diphosphate and by increased production of lactic acid. These hydrogen ions are buffered by bicarbonate present in cells, thereby producing $CO_2$. Thus, increased $dCO_2$ levels are considered to indicate an insufficient circulation.

It is also well known that shock is associated with severe rheological abnormalities of red blood cells (RBC). A recent study reported [14] that RBC deformability was significantly reduced in patients with septic shock as compared with nonseptic patients. In contrast, RBC deformability is not well understood in hemorrhagic shock.

Among models of shock evaluated by intravital microscopy, a rabbit ear chamber (REC) method [15–20] and sidestream dark-field (SDF) imaging [21–23] enable us to observe the microcirculation directly and noninvasively on a real-time basis. The REC method was used in a rabbit model of acute severe hemorrhage to compare the effects of HES and lactated Ringer's solution (LR) on circulatory depression and peripheral microcirculatory dysfunction [16]. We reported [16] that the intravenous infusion of HES 70/0.5 more effectively maintained the peripheral circulation, hemodynamics, and colloidal osmotic pressure than did LR in a rabbit model of acute severe hemorrhage using the REC method.

SDF imaging of the sublingual microcirculation is often used as a bedside monitor to evaluate the microcirculation in patients with shock [22] or to assess the microcirculation in animal models of shock [23]. Recent several studies have shown strong relations between outcomes and cardiovascular dysfunction plus alterations in the sublingual microcirculation [22, 23].

In the present study, we investigate the direct effects of two different solutions, HES 130/0.4 and normal saline solution (NSS), on the rabbit's ear, sublingual microcirculation, $ScvO_2$, $dCO_2$, and RBC deformability after hemorrhagic shock. We focused on these issues to assess the adequacy of fluid resuscitation.

## Materials and methods

### Animal preparation

All experiments were done in accordance with the National Institutes of Health guidelines on the use of experimental animals. Approval from the Animal Use Committee of Tokyo Women's Medical University was obtained (No. AE17-77) before initiating the experiments.

A total of 44 randomly selected Japanese albino rabbits (body weight, 2.5–3.0 kg) were studied. The rabbits were divided into two groups. One group was given NSS ($n = 22$) as control and the other ($n = 22$) was given 6% HES 130/0.4. From each group, eight rabbits were randomly selected to study the REC microcirculation (REC group), eight rabbits to study $ScvO_2$, $dCO_2$ and RBC deformability ($ScvO_2$ group), and six rabbits to study the sublingual microcirculation (Sublingual group).

After intravenous injection of pentobarbital 30 mg/kg, the trachea was intubated in all rabbits, and 1 mg/kg rocuronium was administered intravenously. Anesthesia was maintained with inhaled 0.5% isoflurane with the use of an anesthesia machine (FO-20S; Acoma, Tokyo, Japan). Respiration was controlled with the use of a ventilator (ACE-3000; Acoma, Tokyo, Japan). Arterial blood gases were measured with a portable blood gas analyzer (iSTAT®; ABBOTT JAPAN CO., LTD. Tokyo, Japan) throughout the study. Arterial $PO_2$ ($PaO_2$) was maintained at more than 100 mmHg during the study to eliminate potential effects of systemic hypoxemia on our results. Oral temperature was continuously monitored, and body temperature was maintained constant with the use of a heating pad. Urinary output was continuously monitored. Systolic blood pressure, diastolic blood pressure, and mean arterial pressure (MAP) were monitored with the use of a high-fidelity transducer-tipped catheter (Millar Microtip catheter pressure transducer, 6F SPC-360; Millar Instruments, Houston, TX), placed in the right femoral artery. A similar catheter was placed in the left femoral artery for bloodletting. A 20-G, 3.2-cm catheter (Terumo Co., Tokyo, Japan) was placed in the left auricular vein for infusion of HES or NSS. Blood pressure was monitored continuously using a multichannel polygraph (Model 360; NEC, Tokyo, Japan). After surgical preparation and 30 min of stabilization, baseline hemodynamic variables were recorded.

## Study design and experimental protocol

### Hemorrhagic shock model

To induce shock, bloodletting was performed. The target bleeding volume was equivalent to 40–50% of the circulating blood volume. The bleeding volume per time per minute was 20 mL (10–13% of circulating blood volume). Blood was released a total of four times at 3-min intervals for a total bleeding volume of 80 mL.

In the HES (control) group, 20 mL of 6% HES 130/0.4 (40 mL of NSS) was rapidly infused intravenously 3 min after the bloodletting procedure. Additional HES (NSS) was then administered by intravenous infusion to a total volume of 100 mL (200 mL), delivered at a rate of 160 (320) mL/h for 30 min.

### Assessment of the REC microcirculation (REC group)

Six weeks before the surgical preparation, transparent round chambers made of acrylic resin were inserted into the earlobes of the rabbits as described previously [15]. New microvessels arose from the blood vessels of the dermis and covered the entire cavity within 6 weeks. During the measurements, the RECs were observed microscopically at a magnification of 100×. Microcirculatory changes were recorded using a microscope-closed video camera (DXC 750; Sony, Tokyo, Japan) with a shutter speed of 1/10,000 of a second.

During the stabilization period after surgical preparation, we selected arterioles with diameters of 20–100 μm, displayed on a video television screen. Blood vessel diameter, blood flow velocity, and blood flow rate after the bloodletting and fluid infusion were compared with the baseline values. To analyze blood flow velocity, the play speed of the video recorder was set at 1/60 of a second. The distances between two erythrocytes at the center of the blood vessel were measured ten times, and the values were averaged. Blood flow rate was calculated by multiplying the blood flow velocity by the blood vessel cross-sectional area.

### Measurements of $ScvO_2$, $dCO_2$, and RBC deformability ($ScvO_2$ group)

The jugular vein and femoral artery of the rabbits were dissected and catheterized. Blood was sampled from the jugular vein and femoral artery. A catheter (PediaSat Oximetry Catheter®; Edwards Lifesciences, Irvine, CA, USA) with a size of 4.5 F was placed in the superior vena cava via the jugular vein to monitor $ScvO_2$. $ScvO_2$ was continuously monitored with the use of a Vigileo Monitor® (Edwards Lifesciences, Irvine, CA, USA).

Central venous-to-arterial $CO_2$-gap ($dCO_2$) was calculated from the arterial (a) and central venous (CV) blood gas samples as follows:

$$dCO_2 = PcvCO_2 - PaCO_2.$$

To measure RBC deformability, washed RBCs were suspended in 3.5% PVP solution and subjected to linearly increasing shear stress (0–150 dyn/cm$^2$). Changes in laser diffraction patterns were obtained to derive the deformability index (DI) using an ektacytometer. DI provides a measure of the ellipticity of deforming RBCs in a flow field [24].

### Assessment of the sublingual microcirculation (Sublingual group)

Sublingual microcirculation videos were obtained using a sidestream dark-field imaging device (Microscan®, Microvision Medical, Amsterdam, The Netherlands) [21] derived from an orthogonal polarized spectral imaging technology. The technique is performed using a handheld video microscope and a ring of stroboscopic light-emitting diodes. Light is absorbed by hemoglobin so that RBCs appear dark, yielding a high-contrast video of blood flow in microvessels. Image acquisition and analyses were performed in accordance with the international recommendations, using dedicated analysis software [Automated Vascular Analysis (AVA) version 4.3C; Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands]. The stability of microscan is enhanced by a multiperforated sterile metal ring that adapts to the tip of the sterile cover with probe acquisition. Sublingual microvascular imaging was performed after the bloodletting and fluid infusion, and the subsequent values were compared with the baseline values. We calculated the proportion of perfused vessels [PPV (%)] and perfused vessel density [PVD (vessel/mm$^2$)] using the AVA software.

### Statistical analysis

All data are expressed as means ± SD. Statistical comparisons were performed using Student's *t* test. A value of $P < 0.05$ was considered to indicate statistical significance. Post hoc statistical power was analyzed using G*Power 3.1.9.2 [25]. In the present study, the power is larger than 0.8 for all the results with the significant difference.

## Results

Microscopic examinations with the REC method showed that immediately after blood removal, the mean arteriolar diameter decreased to 45.2 ± 10.8% of the baseline value in

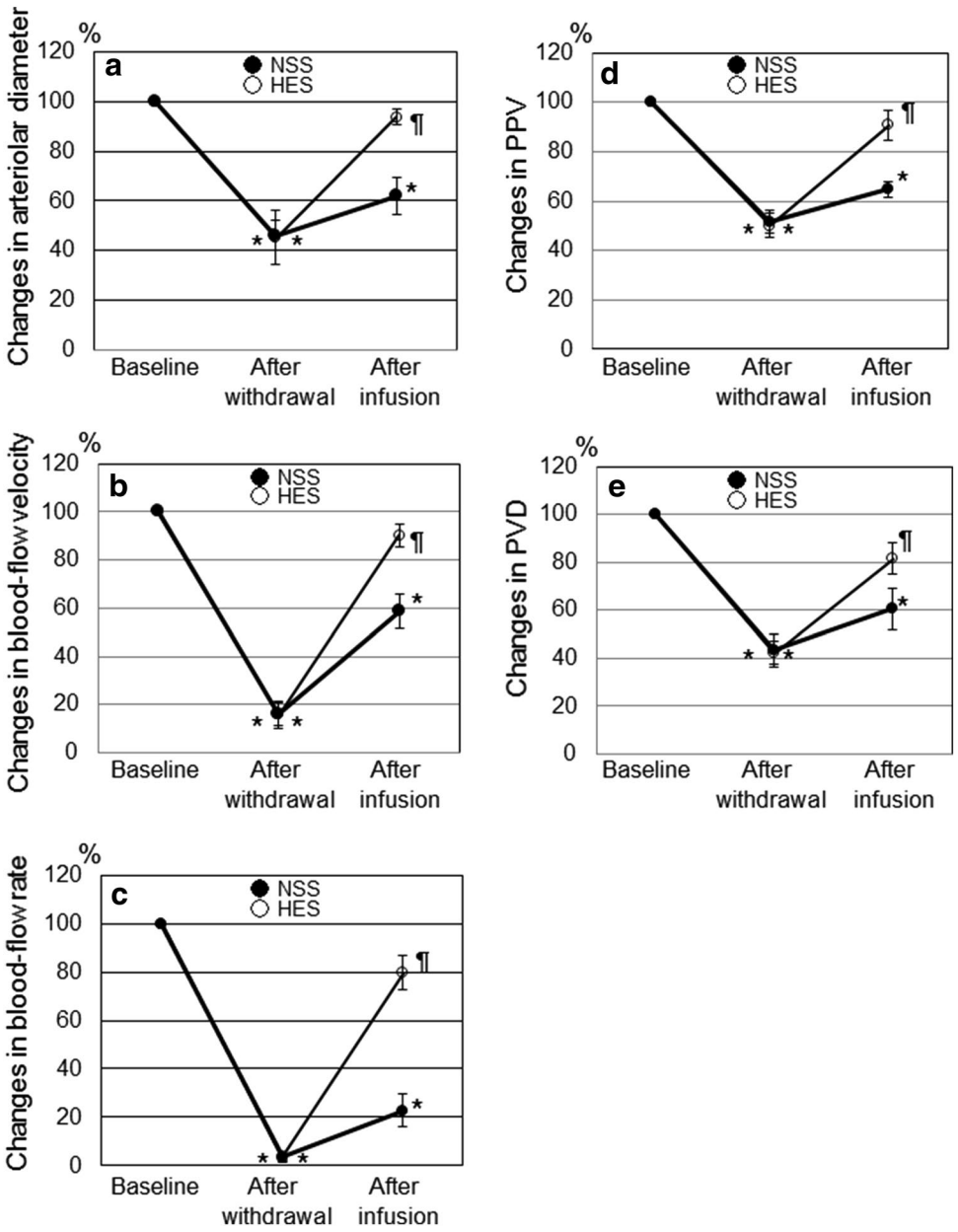


**Fig. 1** Changes in microcirculatory variables after blood withdrawal and infusion therapy. **a** Arteriolar diameter, **b** blood flow velocity, **c** blood flow rate, **d** proportion of perfused vessels (PPV), and **e** perfused vessel density (PVD). Filled circle, normal saline solution (NSS) group; unfilled circle, hydroxyethyl starch (HES) group. *$P < 0.005$ versus the baseline value; ¶$P < 0.005$ versus the NSS group

the HES group as compared with 46.2 ± 5.9% in the NSS group. Immediately after the completion of infusion, the mean arteriolar diameter significantly recovered to 93.9 ± 3.0% of the baseline value in the HES group as compared with 61.9 ± 7.5% in the NSS group ($P < 0.005$; Fig. 1a).

Blood flow velocity and blood flow rate also significantly differed between the groups. Immediately after blood removal, the mean blood flow velocity and mean blood flow rate decreased to 15.8 ± 5.4% and 3.3 ± 1.6% of the respective baseline values in the HES group as compared with 16.1 ± 4.6% and 3.5 ± 1.4% in the NSS group, respectively. After the completion of infusion, mean blood flow velocity and mean blood flow rate significantly recovered to 90.2 ± 4.5% and 79.7 ± 7.2% of each baseline value in the HES group as compared with 58.7 ± 6.9% and 22.9 ± 6.5% in the NSS group ($P < 0.005$; Fig. 1b, c), respectively.

Sublingual microscopic examination showed that immediately after blood removal, changes in PPV decreased to 50.1 ± 4.9% of the baseline value in the HES group compared with 51.7 ± 4.5% in the NSS group. Immediately after the completion of infusion, PPV significantly recovered to 90.1 ± 6.2% of the baseline value in the HES group compared with 64.7 ± 3.3% in the NSS group ($P < 0.005$; Fig. 1d).

Immediately after blood removal, changes in PVD decreased to 42.3 ± 4.6% of the baseline value in the HES group compared with 43.2 ± 6.8% in the NSS group. Immediately after the completion of infusion, PVD significantly

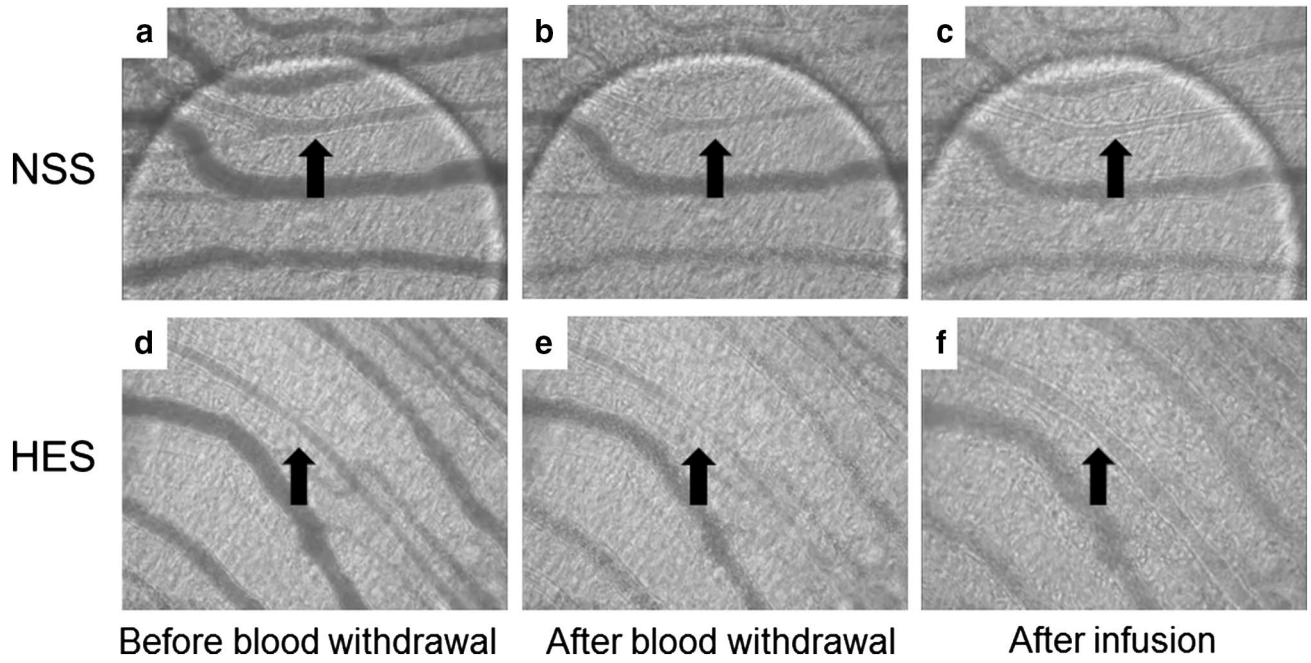


**Fig. 2** Photographs of microvessels of rabbit ear chamber. **a** Before blood withdrawal in the NSS group, **b** after blood withdrawal in the NSS group, **c** after infusion in the NSS group, **d** before blood withdrawal in the HES group, **e** after blood withdrawal in the HES group, and **f** after infusion in the HES group. The arrow indicates an arteriole

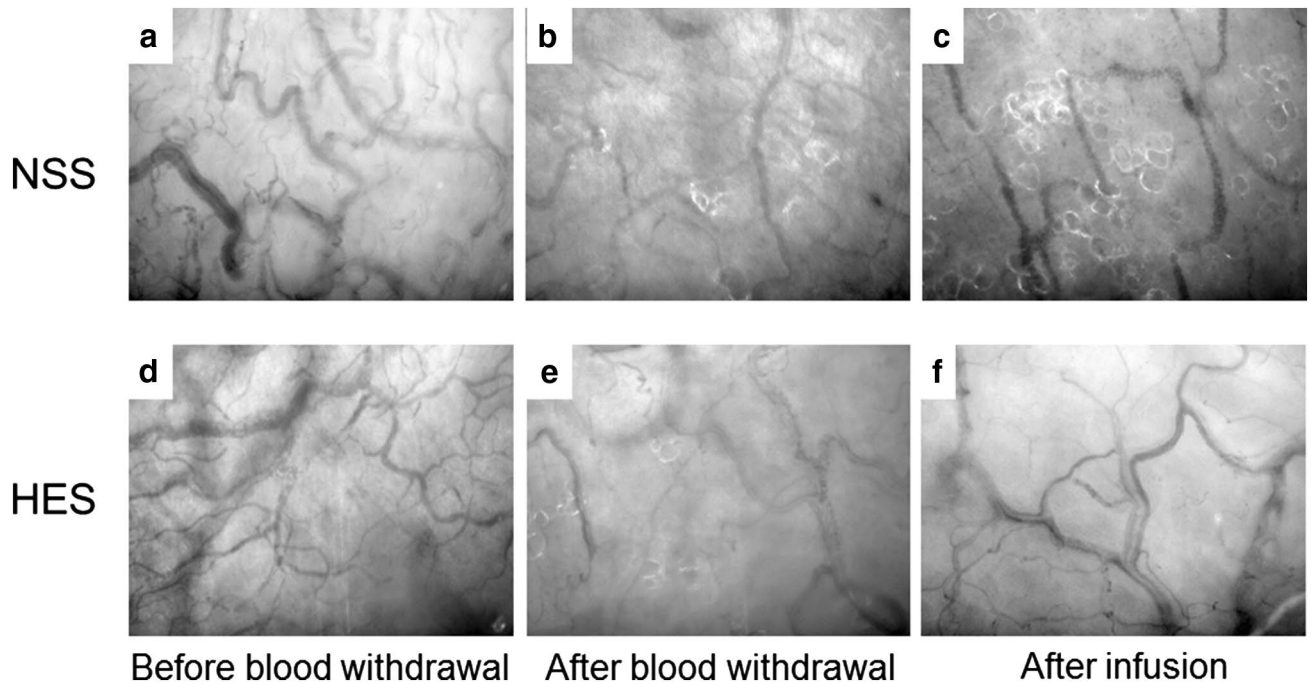


**Fig. 3** Photographs of microvessels of sublingual. **a** Before blood withdrawal in the NSS group, **b** after blood withdrawal in the NSS group, **c** after infusion in the NSS group, **d** before blood withdrawal in the HES group, **e** after blood withdrawal in the HES group, and **f** after infusion in the HES group

recovered to 81.7 ± 6.8% of the baseline value in the HES group as compared with 60.5 ± 8.7% in the NSS group ($P < 0.005$; Fig. 1e).

Microscopic views of microvessels observed with the REC method are shown in Fig. 2. In the both groups, the diameters of the arterioles decreased after blood withdrawal (Fig. 2b, e). After the infusion, the diameters of the arterioles improved (Fig. 2f) in the HES group, but remained poor in the NSS group (Fig. 2c).

Microscopic views of microvessels observed in the sublingual microcirculation are shown in Fig. 3. In the both groups, the capillary density decreased after blood withdrawal (Fig. 3b, e). After the infusion, the capillary density improved (Fig. 3f) in the HES group, but remained poor in the NSS group (Fig. 3c).

MAP at the completion of bloodletting fell to 24.8 ± 4.2% of the baseline value in the HES group and to 26.3 ± 6.7% of the baseline value in the NSS group (Table 1). After the completion of infusion, MAP was equivalent to 96.5 ± 6.3% of the baseline value in the HES group as compared with 70.0 ± 7.1% of the baseline value in the NSS group. The recovery of arterial pressure was significantly better in the HES group than in the NSS group ($P < 0.005$).

$ScvO_2$ at the completion of bloodletting fell to 31.9 ± 15.3% of the baseline value in the HES group and to 32.3 ± 10.5% in the NSS group (Table 1). After the completion of infusion, $ScvO_2$ was equivalent to 87.5% ± 7.9% of the baseline value in the HES group compared with 51.6 ± 4.5% in the NSS group. The recovery of $ScvO_2$ was also significantly better in the HES group than in the NSS group ($P < 0.005$).

After bloodletting, $dCO_2$ increased similarly in the HES group and NSS group (HES, 10.6 ± 2.3 mmHg versus NSS, 9.9 ± 3.5 mmHg). After the completion of infusion, the recovery of $dCO_2$ to its baseline value was significantly better in the HES group than in the NSS group (HES, 5.8 ± 1.3 mmHg versus NSS, 8.9 ± 2.1 mmHg, $P < 0.005$) (Table 1). After bloodletting, the lactate level increased similarly in the HES group and NSS group (HES, 4.8 ± 2.0 mmol/L versus NSS, 4.5 ± 1.9 mmol/L). After the completion of infusion, the recovery of lactate level to its baseline value was significantly better in the HES group than in the NSS group (HES, 1.9 ± 0.7 mmol/L versus NSS, 5.9 ± 2.2 mmol/L, $P < 0.005$) (Table 1).

Mean hematocrit (Hct) after the completion of blood removal and after the completion of infusion was significantly lower than the baseline value in both groups ($P < 0.05$). The Hct after the completion of infusion in the HES group was slightly but not significantly lower than that in the NSS group. The statistical power was 0.54. For $PaO_2$ and temperature, the differences between the groups were insignificant (Table 1).

Table 2 shows changes in MAP, lactate level, hematocrit, $PaO_2$ and oral temperature in each subgroup (REC, $ScvO_2$ and Sublingual groups). There were no significant differences among the subgroups.

Urinary output for an hour was significantly greater in the HES group (7.1 ± 3.2 mL/kg/h) than in the NSS group (3.5 ± 2.4 mL/kg/h; $P < 0.01$, Table 3). As for RBC deformability, maximum DI did not change significantly during the experiments in either group.

## Discussion

The main findings in the present study of the microcirculation in the ear chamber and the sublingual microcirculation assessed with the use of a biomicroscope are summarized as follows. When blood was reperfused after blood removal, the vessel density and perfusion rate of the sublingual microcirculation recovered in the HES group. The

**Table 1** Changes in mean arterial pressure, $ScvO_2$, $dCO_2$, lactate level, hematocrit, $PaO_2$ and oral temperature

| | NSS group | | | HES group | | |
|---|---|---|---|---|---|---|
| | Baseline | After withdrawal | After infusion | Baseline | After withdrawal | After infusion |
| MAP (%) ($n = 22$) | 100 | 26.3 ± 6.7* | 70.0 ± 7.1* | 100 | 24.8 ± 4.2* | 96.5 ± 6.3¶ |
| $ScvO_2$ (%) ($n = 8$) | 100 | 32.3 ± 10.5* | 51.6 ± 4.5* | 100 | 31.9 ± 15.3* | 87.5 ± 7.9¶ |
| $dCO_2$ (mmHg) ($n = 8$) | 5.3 ± 1.5 | 9.9 ± 3.5* | 8.9 ± 2.1* | 5.1 ± 1.4 | 10.6 ± 2.3* | 5.8 ± 1.3¶ |
| Lactate (mmol/L) ($n = 22$) | 1.6 ± 0.6 | 4.5 ± 1.9* | 5.9 ± 2.2* | 1.8 ± 0.6 | 4.8 ± 2.0* | 1.9 ± 0.7¶ |
| Hct (%) ($n = 22$) | 35.5 ± 4.6 | 26.6 ± 6.0* | 20.6 ± 4.8* | 35.0 ± 4.1 | 26.0 ± 4.6* | 16.5 ± 6.4* |
| $PaO_2$ (mmHg) ($n = 22$) | 144 ± 21.7 | 127 ± 20.8 | 112 ± 16.9 | 139 ± 24.2 | 127 ± 21.7 | 118 ± 18.3 |
| Temperature (°C) ($n = 22$) | 37.1 ± 0.8 | 36.8 ± 0.8 | 36.5 ± 0.7 | 37.1 ± 1.0 | 36.6 ± 0.8 | 36.3 ± 0.8 |

All data are expressed as means ± SD

*MAP* mean arterial pressure, *$ScvO_2$* central venous oxygen saturation, *$dCO_2$* central venous-to-arterial carbon dioxide gap. *Hct* hematocrit, *$PaO_2$* arterial $PO_2$

*$P < 0.05$ versus the baseline value

¶$P < 0.005$ versus the NSS group

**Table 2** Changes in mean arterial pressure, lactate level, hematocrit, $PaO_2$ and oral temperature in the subgroups

| | NSS group | | | HES group | | |
|---|---|---|---|---|---|---|
| | Baseline | After withdrawal | After infusion | Baseline | After withdrawal | After infusion |
| REC group | | | | | | |
| MAP (%) ($n=8$) | 100 | 27.4 ± 6.9* | 69.6 ± 9.8* | 100 | 23.3 ± 4.1* | 95.0 ± 5.9¶ |
| Lactate (mmol/L) ($n=8$) | 1.6 ± 0.6 | 4.6 ± 1.3* | 6.5 ± 2.4* | 1.9 ± 0.6 | 4.7 ± 1.5* | 2.0 ± 0.7¶ |
| Hct (%) ($n=8$) | 35.6 ± 4.9 | 26.8 ± 6.4* | 21.0 ± 5.3* | 35.6 ± 4.2 | 27.0 ± 5.3* | 16.3 ± 8.1* |
| $PaO_2$ (mmHg) ($n=8$) | 140 ± 20.4 | 123 ± 17.2 | 108 ± 11.5 | 139 ± 25.0 | 126 ± 21.0 | 118 ± 18.6 |
| Temperature (°C) ($n=8$) | 37.1 ± 0.9 | 36.8 ± 0.8 | 36.4 ± 0.7 | 37.1 ± 1.0 | 36.7 ± 0.8 | 36.3 ± 0.8 |
| $ScvO_2$ group | | | | | | |
| MAP (%) ($n=8$) | 100 | 23.9 ± 5.8* | 66.7 ± 8.2* | 100 | 25.8 ± 5.1* | 97.6 ± 6.4¶ |
| Lactate (mmol/L) ($n=8$) | 1.6 ± 0.7 | 4.5 ± 2.1* | 5.6 ± 1.9* | 1.8 ± 0.6 | 5.3 ± 2.4* | 1.9 ± 0.8¶ |
| Hct (%) ($n=8$) | 34.4 ± 4.6 | 25.0 ± 3.4* | 20.0 ± 3.2* | 34.4 ± 4.4 | 24.9 ± 4.3* | 15.0 ± 5.9* |
| $PaO_2$ (mmHg) ($n=8$) | 148 ± 26.5 | 132 ± 27.1 | 117 ± 24.3 | 135 ± 24.3 | 127 ± 25.0 | 118 ± 19.9 |
| Temperature (°C) ($n=8$) | 36.9 ± 0.8 | 36.8 ± 0.8 | 36.4 ± 0.7 | 37.1 ± 1.1 | 36.6 ± 0.8 | 36.3 ± 0.8 |
| Sublingual group | | | | | | |
| MAP (%) ($n=6$) | 100 | 25.7 ± 3.3* | 71.0 ± 4.3* | 100 | 24.5 ± 2.6* | 97.2 ± 3.8¶ |
| Lactate (mmol/L) ($n=6$) | 1.8 ± 0.7 | 4.5 ± 2.4* | 6.0 ± 2.5* | 1.8 ± 0.6 | 4.5 ± 2.2* | 1.8 ± 0.6¶ |
| Hct (%) ($n=6$) | 36.2 ± 5.5 | 24.8 ± 6.5* | 21.0 ± 5.5* | 35.3 ± 5.8 | 25.8 ± 4.4* | 16.3 ± 6.4* |
| $PaO_2$ (mmHg) ($n=6$) | 145 ± 18.7 | 126 ± 17.7 | 111 ± 11.0 | 143 ± 27.3 | 128 ± 22.1 | 118 ± 19.4 |
| Temperature (°C) ($n=6$) | 37.4 ± 0.8 | 36.9 ± 0.9 | 36.7 ± 0.5 | 37.0 ± 1.1 | 36.5 ± 0.8 | 36.2 ± 0.9 |

All data are expressed as means ± SD

*MAP* mean arterial pressure, *Hct* hematocrit, $PaO_2$ arterial $PO_2$

*$P < 0.05$ versus the baseline value

¶$P < 0.005$ versus the NSS group

**Table 3** Urinary output (mL/kg/h)

| | NSS group | HES group |
|---|---|---|
| All ($n=22$) | 3.5 ± 2.4 | 7.1 ± 3.2** |
| REC group ($n=8$) | 3.3 ± 1.5 | 6.8 ± 2.2** |
| $ScvO_2$ group ($n=8$) | 3.6 ± 1.8 | 7.3 ± 2.9** |
| Sublingual group ($n=6$) | 3.5 ± 2.7 | 7.1 ± 3.3** |

All data are expressed as means ± SD. Urinary output was significantly greater in the HES group (**$P < 0.01$) than in the NSS group for all the groups

arteriolar diameter, blood flow velocity, and blood flow rate of the ear microcirculation were maintained in this group, and microcirculatory failure did not develop. In the NSS group, however, the above five variables were significantly smaller than those in the HES group after the completion of infusion. The recovery of $ScvO_2$ and $dCO_2$ to the respective baseline values was significantly better in the HES group than in the NSS group.

Blood removal causes small blood vessels and capillary vessels to collapse, and blood flow is disrupted. These variables can be restored by HES, while NSS cannot maintain vascular content. Moreover, NSS is associated with increased tissue pressure caused by water leakage to the interstitium, and the vascular lumen narrows, indicating that vessel collapse and vascular disruption progress. In this manner, mass administration of crystalloids not only increases interstitial edema, but also adversely affects the peripheral circulation, including narrowing of the vascular lumen due to endothelial cell edema.

Lowell et al. [26] showed that postoperative weight gain due to intraoperative infusion increased the mortality rate in a dose-dependent manner. The mortality rate was 100% with a weight gain of more than 20%. It is possible that the intraoperative infusion volume can determine the outcome. Besides the mortality rate, excess water volume can cause gastrointestinal dysfunction [27] and increase postoperative nausea, vomiting [28], and the infection rate [29].

Thus, a volume therapy with colloids is recommended as a new type of intraoperative infusion therapy, and the use of crystalline solution is restricted. The third-generation HES 130/0.4 is expected to play an important role in maintaining circulating blood volume. As for vascular permeability, attention has focused on glycocalix. It is known that excessive infusion causes breakdown of glycocalyx and increases vascular permeability. In an electron microscopy study [30], degradation of glycocalyx after hemorrhagic shock was partially restored by plasma, but not by lactated Ringer's solution. Strunden et al. [31] examined how HES modifies the

glycocalyx breakdown of pulmonary microvessels by heparinase in ex vivo experiments in mouse lungs. They showed that HES 130/0.4 acts to maintain the pulmonary circulation via glycocalyx protection. However, a change in glycocalyx due to a large volume of crystalloid has not yet been directly confirmed by electron microscopy.

Regarding the volume of infusion in our study, the amount of HES was 1.25 times of the amount of blood loss, and a half of NSS. The ratio of NSS to HES was fixed according to a previous review by Hartog et al. [7], who concluded it to be 1.8 (SD 0.1) in surgical studies. This is considerably lower than a commonly believed value of 3 or 4 for achieving similar hemodynamic effects. The amount of HES infusion was selected according to a previous report of a study using volunteers by Stand et al. [32]. The volunteers donated 18% of their calculated blood volume within 30 min, and then randomly received 6% HES 130/0.4, 6% HES 70/0.5, or 6% HES 200/0.5 (crossover design) in a 1:1.2 ratio to their blood loss. Then hemodynamic variables, tissue oxygen tension in the quadriceps muscle, hematocrit, plasmatic HES concentrations, plasma viscosity, colloid osmotic pressures, and platelet aggregation were measured after the infusion of HES.

Although the most accurate way to assess cardiac output, oxygen delivery, and consumption is invasive hemodynamic monitoring, it is often unavailable in emergencies. Simple blood gas driven variables such as $ScvO_2$ can help the clinician in defining the need for fluid resuscitation [33]. In our study $ScvO_2$ showed significant changes during hemorrhagic shock and fluid resuscitation. The $ScvO_2$ reflected the actual condition of the REC and sublingual microcirculation. Therefore, $ScvO_2$ may be used as an additional indicator of hemorrhagic shock–resuscitation.

Pathophysiologically, hemorrhagic shock is characterized by systemic microcirculatory failure, which causes anaerobic metabolism. In the HES group, the observed microcirculation variables as well as $dCO_2$ recovered significantly after the completion of infusion. These results suggest that tissue perfusion and oxygenation were restored in the HES group. The $CO_2$ produced by anaerobic metabolism can be washed out when the blood flow is normal or restored [13]. Our results suggest that $dCO_2$ might be a good hemodynamic endpoint of resuscitation. Monitoring $dCO_2$ may be a useful tool for assessing the adequacy of tissue oxygenation during resuscitation.

In the present study, the urine volume was significantly higher in the HES group. It has been reported that the kidney can be damaged by HES [34]. In the CHEST study [35], however, acute kidney damage was infrequently caused by HES. The fluid resuscitation of this study [35] indicated that there is no difference in the mortality between third-generation HES 130/0.4 and physiological saline as a control drug. The previous CRISTAL study [36] compared colloids (HES, albumin, dextran, gelatin) and crystalloids (normal saline, hypertonic saline, lactated Ringer's solution) in patients with hypovolemic shock who were treated in an intensive care unit. The mortality rate was lower in the colloid group, but there was no difference in acute kidney injury or renal replacement therapy between the groups. Linden et al. [37] performed a meta-analysis of tetra-starch (3rd generation HES) and other infusions (albumin, gelatin, dextran, 2nd generation HES, etc.). They showed that there was no difference in the maximum creatinine value or renal replacement therapy rate among these solutions. Konrad et al. [38] showed that the renal microvascular oxygenation in the medulla and cortex during hemodilution with HES 130/0.4 is significantly higher than that during hemodilution with crystalloid solution. The former was concluded to preserve renal function.

In the present study, the arterioles (20–100 μm) were analyzed by the ear chamber method, and the capillary vessels (10–20 μm) and the arterioles (20–100 μm) were analyzed by observation of the sublingual microcirculation. The blood flow of the skin was assessed in the ear. The sublingual microcirculation reflects the blood flow of the cranial region, which is more central than the skin. Therefore, the PPV and PVD of the sublingual microcirculation were less changed than the arteriole blood flow rate of the microcirculation of the skin. A limitation here is that the study of PPV and PVD in the sublingual microcirculation was semiquantitative; identical vessels in the sublingual microcirculation were not observed in contrast to the ear arterioles, and the observed values of PPV and PVD in five fields were merely averaged.

In conclusion, evaluation of the effects of the fluid therapy on the microcirculation, $ScvO_2$, and $dCO_2$ in a rabbit model of hemorrhagic shock showed that the intravenous infusion of HES maintains the peripheral circulation, adequate tissue oxygenation and perfusion better than does NSS infusion.

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## Compliance with ethical standards

**Conflict of interest** The authors declare no conflicts of interest.

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