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Original Article

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Abstract

Resuscitation solution is one of the main interventions provided for septic shock patients, although there is no general consensus regarding the optimal type of solution. Therefore, a trometamol-balanced solution (TBS) was designed as a resuscitation solution. The aim of this study is to assess the effects of TBS versus Ringer's solution (RS) in a rat septic model. Septic shock was induced in 16 male Wistar-Kyoto rats through lipopolysaccharide (LPS) induction; these rats were assigned at a ratio of 1:1 to RS and TBS groups. Blood examinations were performed using an Abbott i-STAT analyzer with CG4+ (for pH, pressure of carbon dioxide, pressure of oxygen, total carbon dioxide, bicarbonate, base excess, oxygen saturation, and lactate) and CG6+ (for sodium, potassium, chloride, blood glucose, blood urea nitrogen, hematocrit, and hemoglobin) and enzyme-linked immunosorbent assay kits (calcium, magnesium, creatinine, aspartate aminotransferase, alanine aminotransferase, bilirubin, and albumin) during the septic state. The biochemical parameters, electrolytes, and blood gas parameters implied similar trends and the majority of data showed no considerable changes between groups after LPS-induced septic shock. However, the pH values of the TBS group were more stable compared with those of the RS group. This indicated that TBS might be more effective in metabolic acidosis alleviation. In summary, TBS is safe and feasible in this study and may offer advantages in septic shock cases without hepatic diseases. Future research will focus on applying TBS in other different animal disease models and the clinical usage of TBS.

Keywords: Fluid resuscitation solution, Ringer's solution, septic model, trometamol-balanced solution

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Introduction

A resuscitation solution is widely considered as the cornerstone of treatment for critically ill patients. Resuscitation solutions can contain crystalloids and colloids (Casey *et al*., 2018). Crystalloid solutions are cheaper and pose a lower risk of infections and anaphylactic reactions compared with colloid solutions. However, in crystalloid solutions, only 20% of the infused volume stays in the vascular space, whereas the remaining volume stays in the interstitial space, inducing tissue edema and organ dysfunction or failure. Nevertheless, determining the ideal resuscitation solution is based on regional variations, clinician experiences, and institute preferences; thus, this topic has been a matter of debate (Finfer *et al*., 2010; Perel and Roberts, 2012; Mutter *et al*., 2013; Lewis *et al*., 2018).

Sepsis and septic shock are major causes of morbidity and mortality in critically ill patients, with the mortality rate being as high as 30%-45% (Dellinger *et al*., 2012; Seymour *et al*., 2016). According to the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3), sepsis is referred to a life-threatening organ dysfunction caused by a dysregulated host response to infection (Singer *et al*., 2016), and septic shock is defined as a subset of sepsis characterized by immune dysregulation, a systemic inflammatory response, microcirculatory abnormalities, and end-organ dysfunction. Therefore, to prevent or treat metabolic acidosis, which is a potential complication of sepsis and septic shock, some strategies have been developed such as the use of the additives L-lactate, acetate, gluconate, and bicarbonate in resuscitation fluids (Dellinger *et al*., 2012; Kimmoun *et al*., 2015; Kraut and Madias, 2016). Acetate and gluconate in trometamol-balanced solution (TBS) are metabolized to bicarbonate, which possesses approximately 53% of the buffering capacity to regulate pH through the consumption of hydrogen cations. Besides of acetate and gluconate, TBS contains trometamol, which is a weak base amino-alcohol that may be superior to bicarbonate for the treatment of acidosis (Nahas *et al*., 1998).

The question concerning the administration of resuscitation fluids during the initial management of septic shock has been the subject of much discussion and debate ever since. Therefore, the purpose of the study is to investigate the effect of using TBS and Ringer's solution (RS) on biochemistry, electrolytes, hematology, and blood gas during the initial resuscitation in a rat septic shock model.

Materials and Methods

Animals: 16 male Wistar-Kyoto rats (age, 8-10 weeks; weighing approximately 250-350 g) were randomly assigned 1:1 to RS and TBS groups and treated with different resuscitation solutions. All rats were housed two per cage in a 12-hr light/dark cycle and were provided free access to Purina chow and water. The experiment was performed at the National Taiwan University (NTU) Laboratory Animal Centre (AAALAC-accredited facility) and approved by the Animal Care and Use Committee of NTU (IACUC number: 20140243).

Resuscitation solutions: According to the Surviving Sepsis Campaign: International Guideline for Management of Severe Sepsis and Septic Shock, it recommends an initial fluid administration in patients with sepsis-induced tissue hypoperfusion to achieve a minimum of 30 mL/kg of crystalloids (Dellinger *et al*., 2012). Therefore, the total volume of resuscitation solutions was 30 mL/kg and was equal between groups. The compositions of the two resuscitation solutions were as follows:

1) RS: pH, 5.8; osmolality, 309 mOsml/kg; Na^+, 147 mmol/L; K^+, 4 mmol/L; Ca^{2+}, 4 mmol/L; and Cl^-, 156 mmol/L (Y F Chemical Corp., Taiwan).
2) TBS: pH, 7.4; osmolality, 282 mOsml/kg; Na^+, 135 mmol/L; K^+, 4 mmol/L; Cl^-, 100 mmol/L; Mg^{2+}, 2 mmol/L; acetate, 24.5 mmol/L; gluconate, 25 mmol/L; and trometamol, 10 mmol/L (Resculyte® solution, Taiwan Biotech Co. Ltd., Taiwan).

Anesthesia, surgical preparation and septic model induction: Details of the anesthetic protocol and surgical procedure were previously prescribed (Ting *et al*., 2020). Anesthesia and surgery were performed by the same team and manner in all rats. Briefly, Sodium pentobarbital (Sigma Chemical Co., USA; 50 mg/kg) was administered intraperitoneally as anesthetic medication. Each rat was oxygenated with ventilator (Model 131, New England Medical Instruments, USA) with 100% oxygen at a tidal volume of 8 ml/kg and a respiratory rate of 70 breath/min. Anesthesia was maintained with sodium pentobarbital intravenous injections (35 mg/kg) each hr. After surgical preparation, lipopolysaccharide (LPS; 60 mg/kg) was injected intraperitoneally to produce inflammation and a shock-like state (Nemzek *et al*., 2008). From pilot study, the LPS induced septic shock in rat model occurred within the first hr after LPS injection and was confirmed by signs noted including reduced motor activity, lethargy and significantly decreased systolic pressure. Therefore, resuscitation solution was administrated through the left femoral vein with the infusion rate of 60 mL/kg/hr within the first 30 min of LPS injection. The fluid resuscitation was finished within the first hr after LPS injection in all rats. Subsequently, the 5-hr study was initiated, and blood samples were obtained every hour. Finally, all rats were humanely euthanized at the end of the study.

Blood sampling: Blood samples at the volume of 0.2-0.5 mL were obtained at the following time points: after surgical preparation (baseline) and 1, 2, 3, 4, and 5 hr after LPS injection (LPS + 1, + 2, + 3, + 4, and + 5 hr, respectively).

Biochemistry, electrolytes, hematology, and blood gas parameters measurement: An Abbott i-STAT analyzer (Abbott Point of Care, USA) was using to analyze the obtained blood samples. Blood samples for the i-STAT CG4+ cartridge (for pH, pressure of carbon dioxide [PCO_2], pressure of oxygen [PO_2] , total carbon dioxide [TCO_2], bicarbonate [HCO_3], base excess [BE], oxygen saturation [sO_2], and lactate) and CG6+ cartridge (for sodium [Na], potassium [K], chloride [Cl], blood

glucose, blood urea nitrogen [BUN], hematocrit [Hct], and hemoglobin [Hgb]) were collected at baseline and LPS + 1, + 2, + 3, + 4, and + 5 hr. Blood samples for determining the levels of total calcium (Ca), magnesium (Mg), creatinine (Cre), aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, and albumin were collected at baseline and LPS + 5 hr; these levels were measured using enzyme-linked immunosorbent assay kits.

Statistical analysis: All statistics were analyzed using the statistical software, R for Mac, version 3.3.2 (The R Foundation). All data are presented as means ± standard deviations. The two-sample *t* test was used to determine differences in parameters between RS and TBS groups from baseline to selected time points. A $p < 0.05$ was considered statistically significant.

Results

A total of 16 rats were included in this study and treated with different resuscitation solutions at the NTU Laboratory Animal Center.

Biochemistry and electrolytes: The biochemistry and electrolyte values in RS and TBS groups were presented in Table 1. The Na level at LPS + 1 hr of the RS group increased from its baseline value, and then the Na levels remained stable. The trend exhibited by the TBS group was similar to that of the RS group. Moreover, the Na level of the TBS group was slightly lower than the RS group. However, all of the Na values were within normal ranges and showed no significant difference between the groups (Fig. 1A). The RS group showed slight hyperchloremia from LPS + 1 hr; then, the Cl level of this group remained stable and over the normal ranges (Fig. 1B). The Cl level of the TBS group also increased from LPS + 1 hr and exhibited a trend similar to that of the RS group. Although the Cl level of the TBS group was slightly lower than that of the RS groups, there was no statistically significant difference between group. The K levels of both groups were gradually increased since the baseline, and peaked at the end of the study; the K level of the RS group seemed to be higher than that of the TBS group (Fig. 1C). However, significant difference between groups was not found.

Table 1 Biochemistry and electrolytes in RS and TBS groups

	Baseline	LPS +1 hour	LPS +2 hours	LPS +3 hours	LPS +4 hours	LPS +5 hours	Normal Ranges
Na (mmol/L)							138-146
RS	135.50±2.39	141.50±2.67	142.00±2.78	142.71±3.45	141.14±2.48	139.20±2.17	
TBS	135.63±4.27	141.75±3.62	141.00±2.67	141.25±1.75	140.57±3.15	139.17±3.37	
K (mmol/L)							3.31-5.50
RS	3.35±0.45	3.64±0.58	3.90±0.58	4.00±0.55	5.14±1.25	5.92±1.40	
TBS	3.38±0.48	3.26±0.48	3.58±0.47	3.90±0.55	4.30±0.56	5.13±1.32	
Cl (mmol/L)							98-107
RS	100.38±3.25	110.00±3.46	111.63±3.02	112.71±4.61	112.29±3.90	111.00±3.08	
TBS	99.88±3.31	108.13±2.10	108.50±3.07	110.25±3.33	110.00±3.83	110.00±2.76	
BUN (mg/dL)							8-26
RS	14.88±2.23	25.00±3.85	30.00±7.35	34.43±5.71	46.57±7.74	53.60±10.16	
TBS	15.75±4.13	23.88±4.76	29.25±5.01	34.25±4.98	39.00±4.32 *	47.83±4.71	
Glucose (mg/dL)							76-175
RS	139.75±30.33	193.13±50.75	163.13±43.69	133.00±63.52	87.00±52.94	63.20±40.27	
TBS	157.13±37.87	216.88±50.61	197.38±46.41	161.50±58.98	123.86±49.56	78.50±38.53	
Lactate (mmol/L)							0.36-1.25
RS	1.22±0.67	4.15±1.15	4.70±1.50	5.29±1.94	6.95±3.53	7.04±2.43	
TBS	0.97±0.13	3.25±1.20	4.16±1.73	5.68±2.50	7.17±4.03	7.45±3.04	
Ca (mg/dL)							9.1-11.2
RS	9.63±1.36					7.88±0.38	
TBS	8.84±0.86					8.58±0.53 *	
Mg (mg/dL)							1.6-2.6
RS	1.86±0.32					3.00±0.51	
TBS	1.70±0.26					3.52±0.80	
Cre (mg/dL)							0.2-0.6
RS	0.19±0.05					0.65±0.26	
TBS	0.18±0.01					0.77±0.22	
AST (U/L)							65-203
RS	60.88±9.83					330.25±42.35	
TBS	58.25±22.05					508.67±247.99	
ALT (U/L)							16-48
RS	39.25±15.82					70.75±30.64	
TBS	36.88±20.93					206.33±126.97*	
Bilirubin (mg/dL)							0.05-0.4
RS	<0.2					<0.2	
TBS	<0.2					<0.2	
Albumin (g/dL)							3.4-5.5
RS	3.19±0.16					2.68±0.10	
TBS	2.79±0.41 *					2.43±0.28	

RS, Ringer's solution; TBS, trometamol-balanced solution; Na, sodium; K, potassium; Cl, chloride; BUN, blood urea nitrogen; Ca, calcium; Mg, magnesium; Cre, creatinine; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LPS, lipopolysaccharide. *$p < 0.05$ between the two groups.

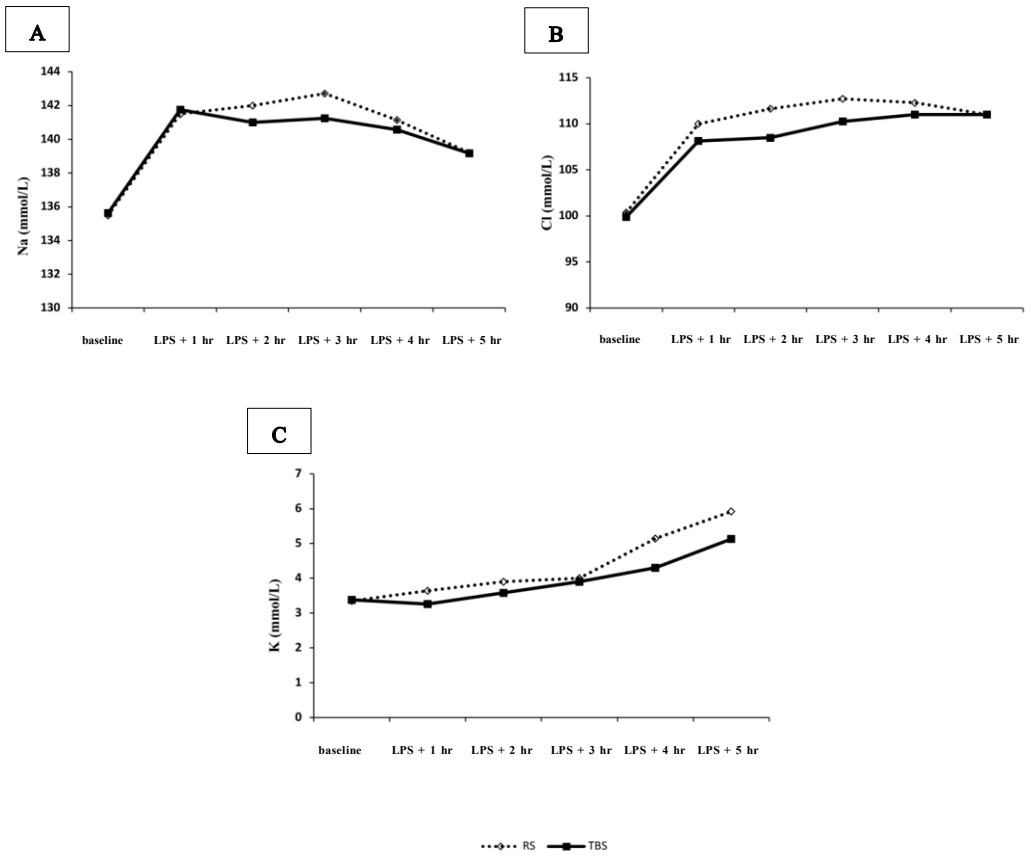

Figure 1 Na (A), Cl (B), and K (C) concentrations at baseline and after LPS-induced septic shock.
RS, Ringer's solution; TBS, trometamol-balanced solution; Na. sodium; Cl, chloride; K, potassium; LPS, lipopolysaccharide.

The blood glucose level peaked at LPS + 1 hr, after which it decreased steeply (Fig. 2A). Neither group exhibited a significant difference in blood glucose level, but the blood glucose level of the RS group was slightly lower than that of the TBS group. Furthermore, the BUN level of both groups gradually rose and was higher than the normal ranges from LPS + 2 hr (Fig. 2B). But only the BUN value of the RS group at LPS + 4 hr was significantly higher than that of the TBS group ($p < 0.05$). Similarly, the lactate level of both groups exhibited an upward trend and increased over the normal range from LPS + 1 hr and finally peaked at LPB + 5 hr (Fig. 2C), but there was no significant difference at any time points.

The Cre, Mg, AST, and ALT levels of both groups all increased from baseline to LPS + 5 hr, exceeding the normal ranges (Fig. 3A-D). Moreover, only the ALT values of the TBS group at LPS + 5 hr was significantly higher than the RS group ($p < 0.05$). By contrast, the Ca and albumin levels of both groups declined from baseline to LPS + 5 hr (Fig. 4A, B) and the Ca level of the TBS group was higher than that of RS group ($p < 0.05$). Furthermore, the bilirubin levels of both groups were measured to be below 0.2 mg/dL during the study and showed no significant difference.

Hematology: The hematology results of RS and TBS groups were shown in Table 2. Hct and Hgb levels of both groups markedly decreased at LPS + 1 hr. Subsequently, these levels remained stable until LPS + 5 hr (Fig. 4C, D). The RS and TBS groups did not exhibit significant differences in Hct and Hgb levels.

Blood gases: The blood gas values of RS and TBS groups were presented in Table 3. The pH value of the blood gases of both groups remained stable from baseline to LPS + 3 hr (Fig. 5A). Subsequently, the pH value of the RS group declined below the normal range from LPS + 4 hr to LPS + 5 hr. By contrast, the pH value of the TBS group declined slightly but was still within the normal range and exhibited significant differences at LPS + 5 hr ($p < 0.05$).

The PCO_2, TCO_2, and HCO_3 levels of both groups showed similar trends and decreased sharply from within the normal ranges at the baselines to below the normal ranges after LPS + 1 hr during the study period (Fig. 5B, D, E). Particularly, the PO_2 level of both groups exhibited an upward trend; normal PO_2 levels were observed before LPS + 3 hr, followed by an increase above 105 mmHg until the end of the study period (Fig. 5C). But no significant differences were noted.

The BE levels of both groups decreased gradually from baseline and were the lowest at LPS + 5 hr (Fig. 5F). The $_SO_2$ level of both groups exhibited an upward trend and remained stable within the normal range (Fig. 5G). However, there was no significant difference between groups at any time point.

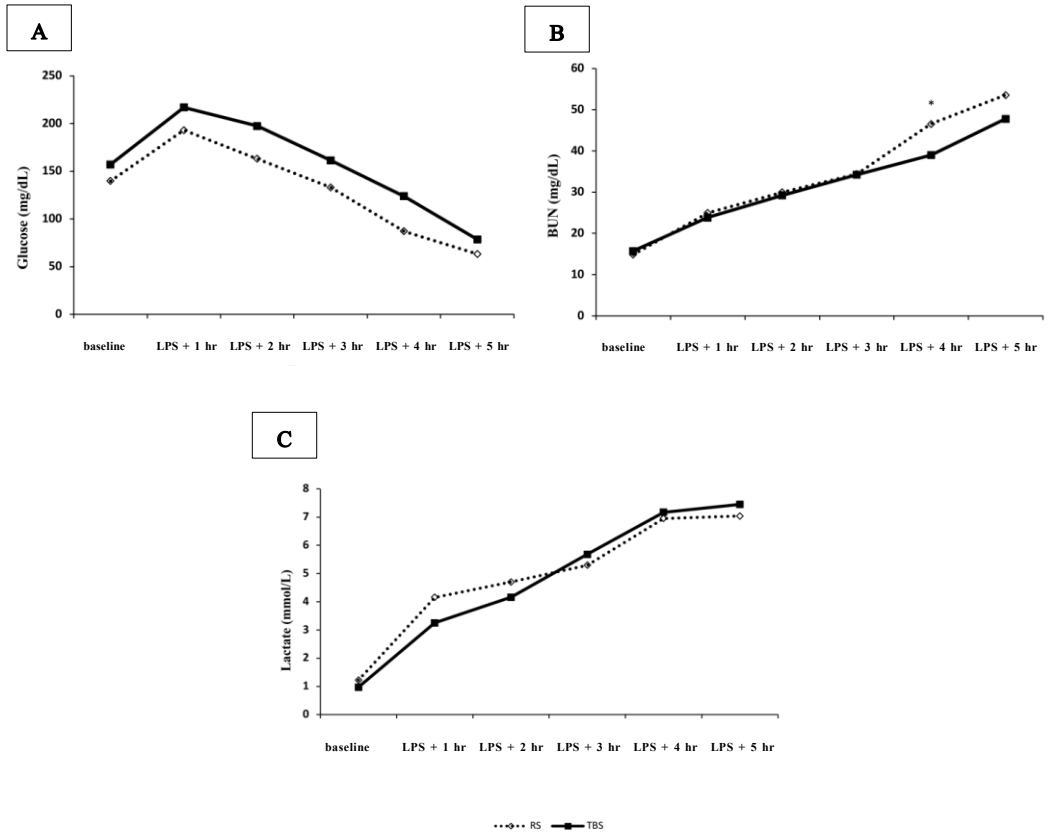

Figure 2 Blood glucose (A), BUN (B), and lactate (C) concentrations at baseline and after LPS-induced septic shock.
RS, Ringer's solution; TBS, trometamol-balanced solution; BUN, blood urea nitrogen; LPS, lipopolysaccharide.
*$p < 0.05$ between the groups.

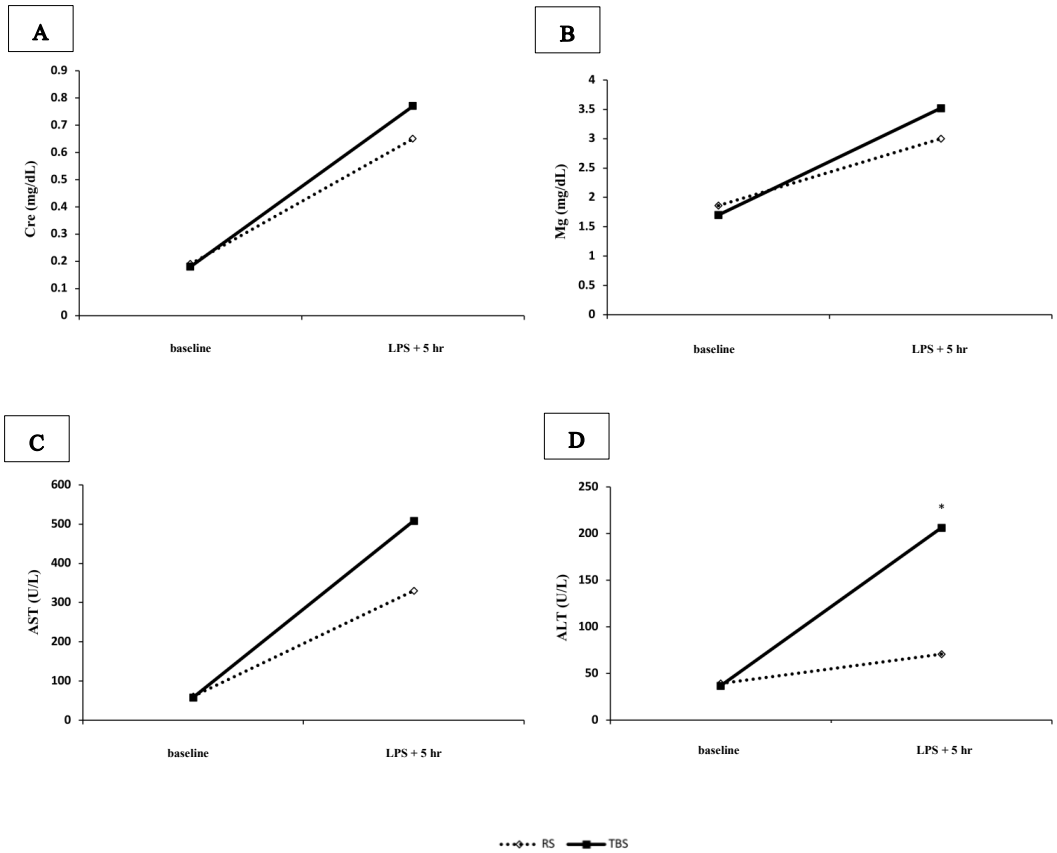

Figure 3 Cre (A), Mg (B), AST (C), and ALT (D) concentrations at baseline and after LPS-induced septic shock.
RS, Ringer's solution; TBS, trometamol-balanced solution; Cre, Creatinine; Mg, magnesium; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LPS, lipopolysaccharide.
*$p < 0.05$ between the groups.

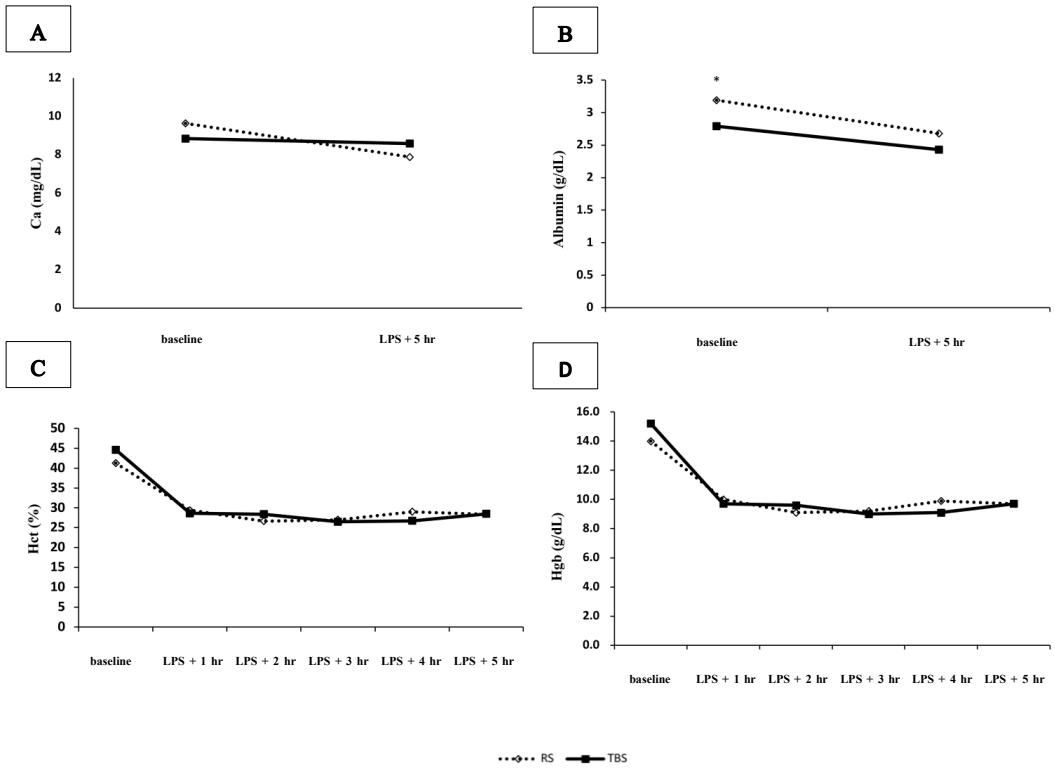

Figure 4 Ca (A), albumin (B), Hct (C) and Hgb (D) concentrations at baseline and after LPS-induced septic shock.
RS, Ringer's solution; TBS, trometamol-balanced solution; Ca, calcium; Hct, hematocrit; Hgb, hemoglobin; LPS, lipopolysaccharide.
*$p < 0.05$ between the groups.

Table 2 Hematology in RS and TBS groups

	Baseline	LPS +1 hour	LPS +2 hours	LPS +3 hours	LPS +4 hours	LPS +5 hours	Normal Ranges
Hct (%)							38-51
RS	41.25±5.39	29.38±6.19	26.63±8.86	27.00±5.16	29.00±6.35	28.40±4.67	
TBS	44.63±2.26	28.63±4.31	28.38±4.34	26.50±5.88	26.71±3.82	28.50±2.66	
Hgb (g/dL)							12-17
RS	14.03±1.84	10.00±2.10	9.11±2.98	9.17±1.76	9.86±2.17	9.68±1.62	
TBS	15.18 ±1.62	9.74±1.46	9.64±1.46	9.01±2.00	9.10±1.30	9.67±0.92	

RS, Ringer's solution; TBS, trometamol-balanced solution; Hct, hematocrit; Hgb, hemoglobin; LPS, lipopolysaccharide.

Table 3 Blood gases in RS and TBS groups

	Baseline	LPS +1 hour	LPS +2 hours	LPS +3 hours	LPS +4 hours	LPS +5 hours	Normal Ranges
pH							7.35-7.45
RS	7.39±0.02	7.39±0.05	7.39±0.04	7.39±0.04	7.33±0.07	7.29±0.07	
TBS	7.38±0.04	7.40±0.04	7.40±0.05	7.40±0.05	7.37±0.07	7.38±0.06 *	
PCO_2 (mmHg)							35-45
RS	38.59±4.46	29.26±4.21	26.86±5.44	25.33±5.67	23.23±7.02	27.06±6.73	
TBS	40.16±4.19	30.19±3.38	26.59±4.29	24.69±6.15	24.23±6.59	21.60±5.59	
PO_2 (mmHg)							80-105
RS	81.13±12.57	91.38±26.84	105.00±34.04	101.14±27.66	106.29±30.35	116.60±38.86	
TBS	83.00±11.88	95.63±16.97	94.38±19.41	101.88±14.22	106.71±22.43	121.00±20.30	
BE (mmol/L)							(-2)-(+3)
RS	-1.38±2.26	-7.38±2.33	-9.00±4.11	-9.86±3.98	-12.86±6.12	-13.40±5.18	
TBS	-1.00±2.27	-6.63±1.19	-8.38±3.78	-9.63±4.03	-10.57±5.88	-11.50±4.68	
HCO_3 (mmol/L)							22-26
RS	23.74±2.21	17.68±1.98	16.18±3.60	15.23±3.79	12.84±5.03	11.70±2.93	
TBS	23.86±2.07	18.43±1.10	16.48±3.11	15.19±3.64	14.50±4.92	13.15±4.05	
TCO_2 (mmol/L)							23-27
RS	24.50±2.88	18.50±2.07	16.88±3.68	16.00±3.87	13.43±5.09	13.80±4.09	
TBS	24.88±2.23	19.25±1.16	17.38±3.25	15.88±3.76	15.14±5.18	13.83±4.40	
sO_2 (%)							95-98
RS	95.38±1.41	96.13±2.23	97.13±1.55	97.00±2.00	97.14±1.57	97.00±1.87	
TBS	95.50±1.69	97.00±1.60	96.63±2.83	97.75±1.28	97.57±1.62	98.67±0.52	

RS, Ringer's solution; TBS, trometamol-balanced solution; PCO_2, pressure of carbon dioxide; PO_2, pressure of oxygen; BE, base excess; HCO_3, bicarbonate; TCO_2, total carbon dioxide;sO_2, oxygen saturation; LPS, lipopolysaccharide.
*$p < 0.05$ between the two groups.

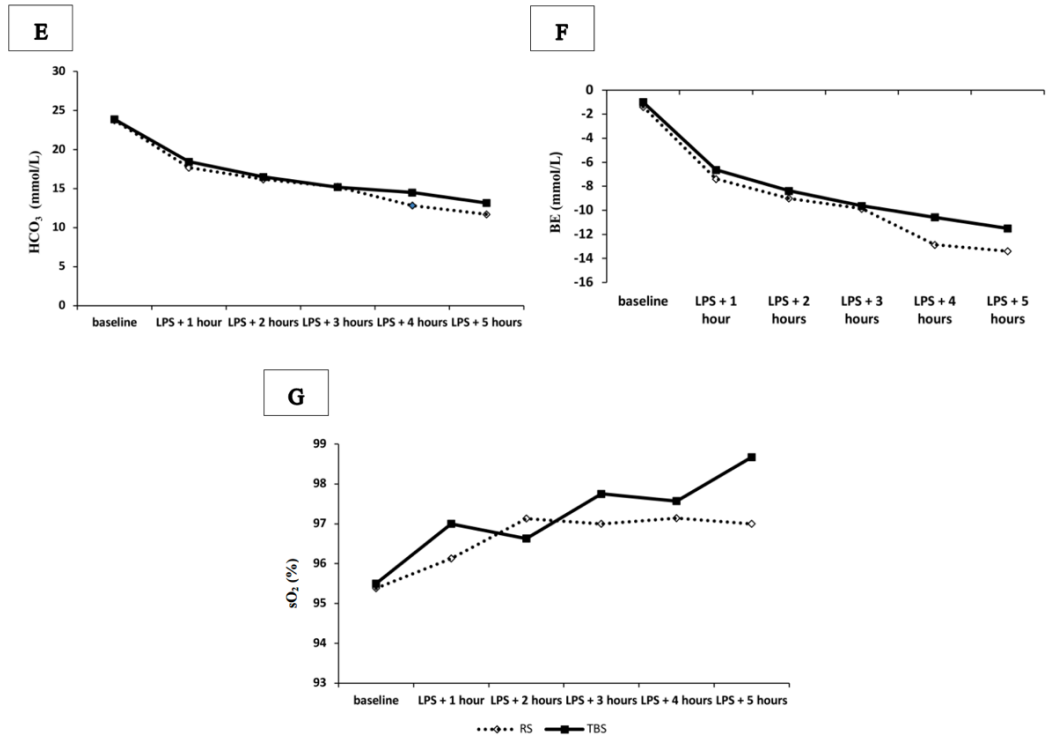

Figure 5 pH (A), PCO_2 (B), PO_2 (C), TCO_2 (D), HCO_3 (E), BE (F), and sO_2 (G) levels at baseline and after LPS-induced septic shock. RS, Ringer's solution; TBS, trometamol-balanced solution; PCO_2, pressure of carbon dioxide; PO_2, pressure of oxygen; TCO_2, total carbon dioxide; HCO_3, bicarbonate; BE, base excess; sO_2, oxygen saturation; LPS, lipopolysaccharide. *$p < 0.05$ between groups..

Discussion

Administration of intravenous resuscitation solution is the most common intervention in the management of septic patients. Despite this, available options for resuscitation solutions vary widely and remain debatable (Casey *et al.,* 2018). Resuscitation solutions are classified as colloid and crystalloid solutions. Colloid solutions efficiently remain in the circulation blood and have a greater intravascular persistence. However, colloid solutions may even induce allergic reactions or increase risk of renal failure and blood clotting disorders (Muttert *et al.,* 2013; Lewis *et al.,* 2018). Compared with colloid solutions, crystalloid solutions are more advantageous because of its lower cost, wider user experience, lower risk of anaphylactic reactions and general availability (Finfer *et al.,* 2010; Muttert *et al.,* 2013; Lewis *et al.,* 2018).

Crystalloid solutions such as RS, lactated RS, and normal saline have been used as resuscitation fluids. RS and normal saline are widely used crystalloid solutions in Taiwan. These solutions are acidic and contain Na and Cl in nearly concentrations, which are higher than physiological levels. Therefore, RS and normal saline are acknowledged to alter the pH values of blood and increase risks of hyperchloremic acidosis and acute kidney injury (Ting *et al.,* 2020). In this study, we designed a new balanced solution, TBS, and assessed the effects of RS and TBS in a rat septic model. TBS contained acetate and gluconate, which are metabolized to bicarbonate by tissue cells and exerted an additional buffering effect to regulate pH. Furthermore, trometamol is the most important component used in TBS. At 37°C, the acid dissociation constant (pK_a) of trometamol is 7.82, making it a more effective buffer than bicarbonate (pK_a = 6.1) in the physiological range of blood pH; therefore, TBS rapidly restores pH and acid-base regulation for alleviating acidosis compared with RS (Nahas *et al.,* 1998; Ting *et al.,* 2020).

***Biochemistry and electrolytes*:** Sepsis and septic shock may result in electrolyte changes through intracellular shift, impaired urination, and renal dysfunction (Velissaris *et al.,* 2015). The Na level of the both groups peaked at LPS + 1 hr and then fluctuated until LPS + 5 hr. The increase in the Na level of both groups may have been due to the administration of sodium-containing solutions and Na retention caused by renal dysfunction (Illner and Shires, 1982). But our data do not exhibit such significant change between groups. The Cl levels of both groups exceeded the normal ranges after LPS injection and remained relatively high until the end of the study and the Cl levels were slightly lower in the TBS group than those of the RS group. However, the K levels of both groups gradually increased after LPS injection, but remained within the normal ranges except for the RS group at LPS + 5 hr. However, neither group exhibited a significant difference in K and Cl levels. This phenomenon of Cl and K levels may be attributed to intracellular shift caused by sepsis-induced metabolic acidosis. Hyperchloremic metabolic acidosis is caused from bicarbonate loss, which occurs in gastrointestinal causes, renal causes, and exogenous causes. The possible factors for hyperchloremia in the study and the higher Cl in the RS group were renal dysfunction and exogenous administration. Renal dysfunction may result from a failure of the distal nephron to secrete hydrogen into the urine or a failure of bicarbonate

reabsorption. In addition, large volume resuscitation leads to an overload of Cl ion into the blood. As for the K levels, sepsis-induced metabolic acidosis, cell electroneutrality is maintained by the movement of intracellular K into the extracellular fluid and results in an elevation of plasma K concentration.

The Mg level was high in both groups, which may be attributable to renal dysfunction caused by sepsis and impaired urination (Baker and Worthley, 2002; Hansen and Bruserud, 2018). In addition, the Mg level at LPS + 5 hr trended to be higher in the TBS group than in the RS group, which might be a result of exogenous Mg administration (TBS contains 2 mmol/L of Mg). But the RS and TBS groups exhibited nonsignificant differences in Mg levels.

The Ca level is tightly regulated by cellular and systemic homeostasis. Ca homeostasis is frequently affected by critical illnesses, such as sepsis and septic shock, and hypocalcaemia has been observed in 88% of critically ill patients (Cumming, 1994; Baker and Worthley, 2002; Kelly and Levine, 2013). In circulation, Ca is found in three different forms of anion-bound, protein-bound and free. The Ca level in each form is dependent on the concentration of hydrogen ions, anions and plasma proteins. A declined pH is associated with decreased Ca binding and therefore increased level of Ca in the free form. Therefore, each 0.1 decrease in pH results in a 0.12 mg/dL increase in serum free Ca concentration (Cumming, 1994; Kelly and Levine, 2013). In addition, each 1 g/dL decrease in albumin levels results in a 0.8 mg/dL increase in serum total Ca concentration (Cumming, 1994; Kelly and Levine, 2013). The total Ca level of the TBS group was higher than that of the RS group and remained within the normal ranges at LPS + 5 hr. It may result from the relatively stable pH value and albumin level in the TBS group at LPS + 5 hr. Moreover, a significant difference in the Ca level was observed between the groups at LPS + 5 hr ($p < 0.05$). Studies have suggested treating hypocalcaemia through parenteral administration of Ca supplements (Collage *et al.,* 2013; Kelly and Levine, 2013). Nevertheless, no beneficial evidence in animal model experiments has been reported to approve the treatment (Collage *et al.,* 2013; Dotson *et al.,* 2016). Therefore, Ca was not added in TBS.

The blood glucose level increased from baseline and peaked at LPS + 1 hr. Hyperglycemia may be caused by the release of cytokines and catecholamines after an inflammatory response following LPS injection (Illner and Shires, 1982; Baker and Worthley, 2002; Velissaris *et al.,* 2015). In addition to the inflammatory response, pain and an anesthesia-induced stress response might engender increased blood glucose levels (Miller *et al.,* 1980; Thompson, 2008). However, in this study, the blood glucose level declined gradually from LPS + 2 hr, and a hypoglycemic state was reached at LPS + 5 hr. These findings suggest that hypoglycemia can be caused by septic shock. Besides septic shock, trometamol has been recognized to increase insulin secretion and therefore lower blood glucose. But there were no significantly differences among all groups in the study.

Lactate levels are indicators of poor tissue perfusion and can be influenced by many factors, including hypothermia, extreme hemodilution, low flow of cardiopulmonary bypass, and excessive neurohormonal activation (Chertoff *et al.,* 2015; Lee and An, 2016). Increased serum lactate level in the progressive sepsis is frequently regarded as evidence of tissue hypoxia or oxygen debt secondary to hypoperfusion. Therefore, sepsis-associated hyperlactatemia (SAHL) is due to anaerobic glycolysis induced by tissue hypoxia which is widely believed to be an important cause of organ failure and mortality. According to the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3), patients with a serum lactate level of >2 mmol/L can be diagnosed with septic shock (Shankar-Hari *et al.,* 2016). Therefore, hyperlactatemia observed in RS and TBS groups after baseline in this study was caused by LPS injection. The Surviving Sepsis Campaign: International Guideline for Management of Severe Sepsis and Septic Shock recommend an initial fluid load with 30 mL/kg of crystalloids for septic patients presenting tissue hypoperfusion, hypotension or signs of hypovolemia (Dellinger *et al.,* 2012). Furthermore, antibiotic therapy, vasopressors, inotropes and red blood cells transfusion are also the therapeutic interventions available for clinicians to achieve those hemodynamic goals. But in our study, the rats were only received resuscitation solutions which was not able to control the metabolic acidosis and organ dysfunction by septic shock. Therefore, the lactate level did not improve in either groups and increased gradually with the time points. Nevertheless, both groups exhibited similar trends with no significant changes in the lactate level.

Acute kidney injury (AKI) is a common problem in sepsis and septic shock patients (Majumdar, 2010; Zarjou and Agarwal, 2011). The pathophysiology of sepsis-induced AKI is multifactorial and complex and can involve endothelial dysfunction, inflammatory cell infiltration, hemodynamic changes, and intraglomerular thrombosis (Wan *et al.,* 2008; Bagshaw *et al.,* 2009). The BUN and Cre levels of both groups exhibited obvious renal dysfunction after LPS injection. Moreover, except for the BUN level at LPS + 4 hr, the BUN level did not significantly differ between the groups.

In sepsis and septic shock, liver injury is caused by bacteria, toxins, or inflammatory mediators with progression from hepatocellular dysfunction to liver damage and finally to liver failure (Crouser *et al.,* 2008; Strnad *et al.,* 2017). Therefore, the AST and ALT levels of both groups increased from normal ranges at the baseline to peaks at the end of the study. Notably, the higher AST and ALT levels in the TBS group than in the RS group may be resulted from the presence of trometamol in TBS, which could have increased the loading of hepatic metabolites. In addition, the albumin level in both groups was lower at LPS + 5 hr than that of baseline. Hypoalbuminemia is frequently observed and it can be associated with several diseases, such as cirrhosis, malnutrition and sepsis (Takegawa *et al.,* 2019). Hypoalbuminemia can be divided into four factors, including decreasing synthesis, increasing loss, redistributing albumin and albumin diluting. In this study, administering 30 mL/kg of resuscitation solutions may be attributed to hypoalbuminemia by dilution of all constituents of whole blood.

Furthermore, blood samples collected may also play a minor factor to decrease albumin levels. As for the factor of poor liver function, decreased serum albumin levels are not seen in acute liver failure because it takes several weeks of impaired albumin production before the serum albumin level drops.

In sepsis and septic shock, hyperbilirubinemia is a common complication associated with liver dysfunction (Muftuoglu *et al.,* 2006; Strnad *et al.,* 2017). However, the bilirubin level in both the groups was measured to be below 0.2 mg/dL during the study. Animal sepsis models have demonstrated a significant conjugation defect with elevated unconjugated bile acid levels after 15 hours of the initiation of sepsis (Muftuoglu *et al.,* 2006; Woźnica *et al.,* 2018). Therefore, the bilirubin level in both groups was within the normal range in 5 hours of the study. But the bilirubin levels are more likely to rise after a while.

***Hematology*:** Anemia is very common in acutely ill patients with sepsis and septic shock. The etiology of anemia is multifactorial and involves blood sampling, blood loss, decreased red blood cell (RBC) synthesis, and increased RBC destruction by inflammatory mediators (Hayden *et al.,* 2012; Straat *et al.,* 2012; Bateman *et al.,* 2017). Nevertheless, anemia caused by sepsis and septic shock is poorly explained in the study. The Hct and Hgb levels of both groups were lower after LPS-injection than those at baseline; this finding may be attributed to hemodilution resulting from abundant intravenous infusions. A rat has a circulating blood volume of approximately 5.5%-7% of body weight (Lee and Blaufox, 1985), and in this study, LPS injection and resuscitation fluids were administered according to 3.6% of body weight. This is the reason for lower Hct and Hgb levels after baseline. Furthermore, blood samples were obtained 6 times and 0.2-0.5 mL of blood required for each time point testing. The totally maximum blood sample volume was 3 mL (about 0.85-1.2 % body weight) which was a possible factor responsible for lower Hct and Hgb levels in this study, although the sampling volume was low. No differences in hematological parameters were observed between the TBS and RS groups.

Blood gases: Sepsis and septic shock induce metabolic acidosis, which is associated with the acid-base balance in various organ dysfunctions (Finfer *et al.,* 2010; Perel and Roberts, 2012). To prevent and alleviate acidosis, TBS was designed as a neutralizing solution by using trometamol. After LPS injection, the pH values were found to be lower than 7.40, and BE and HCO_3 levels were determined to be lower than normal ranges, indicating metabolic acidosis. This phenomenon may be caused by poor tissue perfusion in sepsis and septic shock, although, each rat was oxygenated with ventilator with 100% oxygen at a tidal volume of 8 mL/kg and a respiratory rate of 70 breath/min. Notably, the pH value of the TBS group was higher and more stable than that of the RS group. The stable pH value of the TBS group can be attributed to trometamol, which is a weak base amino-alcohol that may have a superior buffering capacity for the treatment of acidosis compared with RS (Finfer *et al.,* 2010; Dellinger *et al.,* 2012; Kimmoun *et al.,* 2015; Kraut and Madias, 2016).

In summary, the RS and TBS groups exhibited similar alternations of biochemistry, electrolytes, hematology and blood gas without significant differences in the study. Compared with RS, TBS showed the potential to alleviate metabolic acidosis during septic shock. However, patients with pre-existing hepatic diseases require careful assessment of liver function in order to avoid hepatic impairment by the trometamol, acetate, and gluconate in TBS. To conclude, TBS seems to be safe and possible for use as a resuscitation solution in rats, but an area of future research that should be considered is the different parameters, and the clinical application of swine and companion animals.

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References

Bagshaw SM, Lapinsky S, Dial S, Arabi Y, Dodek P, W ood G, Ellis P, Guzman J, Marshall J, Parrillo JE, Skrobik Y, Kumar A and Cooperative Antimicrobial Therapy of Septic Shock (CATSS) Database Research Group 2009. Acute kidney injury in septic shock: clinical outcomes and impact of duration of hypotension prior to initiation of antimicrobial therapy. Intensive Care Med. 35: 871-881.

Baker SB and Worthley LIG 2002. The essentials of calcium, magnesium and phosphate metabolism: Part II. Disorders. Crit Care Resusc. 4: 307-315.

Bateman RM, Sharpe MD, Singer M and Ellis CG 2017. The effect of Sepsis on the Erythrocyte. Int J Mol Sci. 18: 1932.

Casey JD, Brown RM and Semler MW 2018. Resuscitation fluids. Curr Opin Crit Care. 24: 512-518.

Chertoff J, Chisum M, Garcia B and Lascano J 2015. Lactate kinetics in sepsis and septic shock: a review of the literature and rationale for further research. J Intensive Care. 3: 39.

Collage RD, Howell GM, Zhang X, Stripay JL, Lee JS, Angus DC and Rosengart MR 2013. Calcium supplementation during sepsis exacerbates organ failure and mortality via calcium/calmodulin-dependent protein kinase kinase (CaMKK) signaling. Crit Care Med. 41: e352-60.

Crouser E, Exline M, Knoell D and Wewers MD 2008. Sepsis: links between pathogen sensing and organ damage. Curr Pharm. 14: 1840-1852.

Cumming AD 1994. Changes in plasma calcium during septic shock. J Accid Emerg Med. 11: 3-7.

Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, Sevransky JE, Sprung CL, Douglas IS, Jaeschke R, Osborn TM, Nunnally ME, Townsend SR, Reinhart K, Kleinpell RM, Angus DC, Deutschman CS, Machado FR, Rubenfeld GD, Webb S, Beale RJ, Vincent JL, Moreno R and

Surviving Sepsis Campaign Guidelines Committee including The Pediatric Subgroup 2013. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock, 2012. Intensive Care Med. 39: 165-228.

Dotson B, Larabell P, Patel J, Wong K, Qasem L, Arthur W, Leiberman C, Whittaker P and Tennenberg SD 2016. Calcium administration is associated with adverse outcomes in critically ill patients receiving parenteral nutrition: results from a natural experiment created by a calcium gluconate shortage. Pharmacotherapy. 36: 1185-1190.

Finfer S, Liu B, Taylor C, Bellomo R, Billot L, Cook D, Du B, McArthur C, Myburgh J and SAFE TRIPS Investigators 2010. Resuscitation fluid use in critically ill adults: an international cross sectional study in 391 intensive care units. Crit Care. 14: R185.

Hansen BA and Bruserud Ø 2018. Hypomagnesemia in critically ill patients. J Intensive Care. 6: 21.

Hayden SJ, Albert TJ, Watkins TR and Swenson ER 2012. Anemia in critical illness: insights into etiology, consequences, and management. Am J Respir Crit Care Med. 185: 1049-1057.

Illner H and Shires GT 1982. Changes in sodium, potassium, and adenosine-triphosphate contents of red-blood-cells in sepsis and septic shock. Circ Shock. 9: 259-267.

Kelly A and Levine MA 2013. Hypocalcemia in the critically ill patient. J Intensiv Care Med. 28: 166-177.

Kimmoun A, Novy E, Auchet T, Ducrocq N and Levy B 2015. Hemodynamic consequences of severe lactic acidosis in shock states: from bench to bedside. Crit Care. 19: 175.

Kraut JA and Madias NE 2016. Lactic acidosis: current treatments and future directions. Am J Kidney Dis. 68: 473-482.

Lee HB and Blaufox MD 1985. Blood volume in the rat. J Nucl Med. 26: 72-76.

Lee SM and An WS 2016. New clinical criteria for septic shock: serum lactate level as new emerging vital sign. J Thorac Dis. 8: 1388-1390.

Lewis SR, Pritchard MW, Evans DJ, Butler AR, Alderson P, Smith AF and Roberts I 2018. Colloids versus crystalloids for fluid resuscitation in critically ill people. Cochrane Database Syst Rev. 8: CD000567.

Majumdar A 2010. Sepsis-induced acute kidney injury. Indian J Crit Care Med. 14: 14-21.

Miller SI, Wallace RJ Jr, Musher DM, Septimus EJ, Kohl S and Baughn RE 1980. Hypoglycemia as a manifestation of sepsis. Am J Med. 68: 649-654.

Muftuoglu MA, Aktekin A, Ozdemir NC and Saglam A 2006. Liver injury in sepsis and abdominal compartment syndrome in rats. Surg Today. 36: 519-524.

Mutter TC, Ruth CA and Dart AB 2013. Hydroxyethyl starch (HES) versus other fluid therapies: effects on kidney function. Cochrane Database Syst Rev. 7: CD007594.

Nahas GG, Sutin KM, Fermon C, Streat S, Wiklund L, Wahlander S, Yellin P, Brasch H, Kanchuger M, Capan L, Manne J, Helwig H, Gaab M, Pfenninger E, Wetterberg T, Holmdahl M and Turndorf H 1998. Guidelines for the Treatment of Acidaemia with THAM. Drugs. 55: 191-224.

Nemzek JA, Hugunin KM and Opp MR 2008. Modeling sepsis in the laboratory: Merging sound science with animal well-being. Comp Med. 58: 120-128.

Perel P and Roberts I 2012. Colloids versus crystalloids for fluid resuscitation in critically ill patients. Cochrane Database Syst Rev. 6: CD000567.

Seymour CW, Liu VX, Iwashyna TJ, Brunkhorst FM, Rea TD, Scherag A, Rubenfeld G, Kahn JM, Shankar-Hari M, Singer M, Deutschman CS, Escobar GJ and Angus DC 2016. Assessment of clinical criteria for sepsis: for the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 315: 762-774.

Shankar-Hari M, Phillips GS, Levy ML, Liu VX, Deutschman CS, Angus DC, Rubenfeld GD, Singer M and Sepsis Definitions Task Force 2016. Developing a new definition and assessing new clinical criteria for septic shock: for the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 315: 775-787.

Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, Bellomo R, Bernard GR, Chiche JD, Coopersmith CM, Hotchkiss RS, Levy MM, Marshall JC, Martin GS, Opal SM, Rubenfeld GD, van der Poll T, Vincent JL and Angus DC 2016. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 315: 801-810.

Straat M, van Bruggen R, de Korte D and Juffermans NP 2012. Red blood cell clearance in inflammation. Transfus Med Hemother. 39: 353-361.

Strnad P, Tacke F, Koch A and Trautwein C 2017. Liver-Guardian, modifier and target of sepsis. Nat Rev Gastroenterol Hepatol. 14: 55-66.

Takegawa R, Kabata D, Shimizu K, Hisano S, Ogura H, Shintani A and Shimazu T 2019. Serum albumin as a risk factor for death in patients with prolonged sepsis: An observational study. J Crit Care. 51: 139-144.

Thompson BT 2008. Glucose control in sepsis. Clin Chest Med. 29: 713-720.

Ting WT, Chang RW, Wang CH, Chen YS and Lee JJ 2020. Comparison of the trometamol-balanced solution with two other crystalloid solutions for fluid resuscitation of a rat hemorrhagic model. J Vet Sci. 21: e6.

Velissaris D, Karamouzos V, Pierrakos C, Aretha D and Karanikolas M 2015. Hypomagnesemia in critically Ill sepsis patients. J Clin Med Res. 7: 911-918.

Wan L, Bagshaw SM, Langenberg C, Saotome T, May C and Bellomo R 2008. Pathophysiology of septic acute kidney injury: what do we really know? Crist Care Med. 36: S198-S203.

Woźnica EA, Inglot M, Woźnica RK and Łysenko L 2018. Liver dysfunction in sepsis. Adv Clin Exp Med. 27: 547-551.

Zarjou A and Agarwal A 2011. Sepsis and acute kidney injury. J Am Soc Nephrol. 22: 999-1006.