Effects of goal-directed fluid therapy on microcirculation during human study and animal experiment

PhD thesis

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LIST OF PUBLICATIONS

LIST OF FULL PAPERS RELATED TO THE SUBJECT OF THE THESIS

I. László I**,** Demeter G, Öveges N, Érces D, Kaszaki J, Tánczos K, Molnár Z. Volume-replacement ratio for crystalloids and colloids during bleeding and resuscitation: an animal experiment. Intensive Care Med Exp. 2017 Dec 20;5(1):52. **IF: 0.000**

II. **László I**, Janovszky Á, Lovas A, Vargán V, Öveges N, Tánczos T, Mikor A, Trásy D, Lóderer Z, Piffkó J, Szabó A, Molnár Z. Effects of goal-directed crystalloid vs. colloid fluid therapy on microcirculation during free flap surgery: A randomised clinical trial. Eur J Anaesthesiol. 2019 Aug; 36(8):592-604. **IF: 4.500**

III. **László I**, Janovszky Á, Szabó A, Molnár Z. "Reply to: Crystalloids Should Be Second Choice for Goal-Directed Fluid Therapy." Eur J Anaesthesiol. 2020 May;37(5):415-416.

IV. **László I,** Janovszky Á, Szabó A, Molnár Z. Reply to: effects of goal-directed crystalloid vs. colloid fluid therapy on microcirculation during free flap surgery. Eur J Anaesthesiol. 2020 May; 37(5):413-414.

LIST OF BOOK CHAPTERS RELATING TO THE SUBJECT OF THE THESIS

V. **Ildikó, László** ; Nándor, Öveges ; Zsolt M. Distribution of Crystalloids and Colloids During Fluid Resuscitation: All Fluids Can be Good and Bad? In: Annual Update in Intensive Care and Emergency Medicine, Springer International Publishing. 2017, 91–103.

LIST OF ABSTRACTS RELATING TO THE SUBJECT OF THE THESIS

VI. **László** I, Demeter G, Öveges N, Tánczos K, Németh M, Trásy D, Kertmegi I, et al. 2016. "Physiological Volume Replacement Ratio Can Be Reached in Experimental Hemorrhage Model." INTENSIVE CARE MEDICINE 4 (Suppl.1.): 89–90.

VII. **László I**., Janovszky Á, Öveges N, Lóderer Z, József P, Szabó A, Vargán V, et al. 2018. "Effects of Crystalloid vs. Colloid Volume Replacement on Microcirculatory Perfusion in Free Flap Surgery." CRITICAL CARE 22 (Suppl. 1.): 126–127.

OTHER PAPERS NOT RELATED TO THE SUBJECT OF THE THESIS

VIII. Zöllei E, Bertalan V, Németh A, Csábi P, **László** I, Kaszaki J, Rudas L. Non-invasive detection of hypovolemia or fluid responsiveness in spontaneously breathing subjects. BMC Anesthesiol. 2013 Nov 5;13(1):40. **IF: 1.333**

IX. **László** I, Trásy D, Molnár Z, Fazakas J. Sepsis: From Pathophysiology to Individualized Patient Care. J Immunol Res. 2015;2015:510436. **IF: 2.934**

X. Tánczos K, Németh M, Trásy D, **László** I, Palágyi P, Szabó Z, Varga G, Kaszaki J Goal-Directed Resuscitation Aiming Cardiac Index Masks Residual Hypovolemia: An Animal Experiment. Biomed Res Int. 2015;2015:160979. **IF: 2.134**

XI. Palágyi P, Barna S, Csábi P, Lorencz P, **László** I, Molnár Z. Recent Advances of Mucosal Capnometry and the Perspectives of Gastrointestinal Monitoring in the Critically Ill. A Pilot Study. J Crit Care Med (Targu Mures). 2016 Feb 9;2(1):30-37. **IF: 0.000**

XII. Trásy D, Tánczos K, Németh M, Hankovszky P, Lovas A, Mikor A, **László** I, Hajdú E, Osztroluczki A, Fazakas J, Molnár Z Early procalcitonin kinetics and appropriateness of empirical antimicrobial therapy in critically ill patients: A prospective observational study. EProK study group. J Crit Care. 2016 Aug;34:50-5. **IF: 2.648**

XIII. Kaszaki, József, **Ildikó László**, László Juhász, and Andrea Szabó. 2016. "Szepszis - Mikrokeringéstől a Mitokondriumokig [Sepsis - from the Microcirculation to the Mitochondial Function]." ANESZTEZIOLÓGIA ÉS INTENZÍV TERÁPIA 47 (1): 15–22. **IF:0.000**

XIV. Ruszkai Z, Kiss E, **László** I, Gyura F, Surány E, Bartha PT, Bokrétás GP, Rácz E, Buzogány I, Bajory Z, Hajdú E, Molnár Z. Effects of intraoperative PEEP optimization on postoperative pulmonary complications and the inflammatory response: study protocol for a randomized controlled trial. Trials. 2017 Aug 11;18(1):375. **IF: 2.067**

XV. Öveges N, **László** I, Tánczos K, Németh M, Lebák G, Tudor-Drobjewski BA, Érces D, Kaszaki J, Rudas L, Huber W, Molnár Z. Mean arterial pressure targeted fluid resuscitation may lead to fluid overload: A bleeding-resuscitation animal experiment. PLoS One. 2018 Jun 28;13(6):e0196188. **IF: 2.776**

XVI. Hawchar F, **László** I, Öveges N, Trásy D, Ondrik Z, Molnar Z. Extracorporeal cytokine adsorption in septic shock: A proof of concept randomized, controlled pilot study. J Crit Care. 2019 Feb;49:172-178. **IF: 2.685**

XVII. Ruszkai Z, Kiss E, **László** I, Bokrétás GP, Vizserálek D, Vámossy I, Surány E, Buzogány I, Bajory Z, Molnár Z. Effects of intraoperative positive end-expiratory pressure optimization on respiratory mechanics and the inflammatory response: a randomized controlled trial. J Clin Monit Comput. 2021 May;35(3):469-482. **IF: 1.977**

XVIII. **László, Ildikó** ; Csákány, Lóránt* ; Veres, Antal ; Németh, Márton ; Poles, Marietta ; Kaszaki, József ; Molnár, Zsolt ; Szabó, Andrea Az endotheliális glycocalyx megjelenítése és vastagságának meghatározása intravitális mikroszkópiával. ANESZTEZIOLÓGIA ÉS INTENZÍV TERÁPIA 51 : 4 pp. 11-20. , 10 p. (2021) **IF:0.000**

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SUMMARY OF THE THESIS

Fluid resuscitation remains one of the cornerstone's in the management of acute bleeding. According to Starling's "Three-compartment model", four-times more crystalloids (Cryst) have the same volume-replacement (VR) effect as colloids (Coll). However, this VR ratio remains a controversial issue as it may be affected by the degradation of the endothelial glycocalyx layer (GX), a situation often found in the critically ill. On the other hand, macro- and microcirculatory effects of solutions are difficult to compare, because the endpoints of resuscitation seldom based on similar criteria.

Our aims were: (1) to compare the effects of Coll and Cryst based fluid resuscitation during an experimental stroke volume index (SVI) guided haemorrhage and resuscitation animal model; (2) to investigate the effects of Cryst and Coll's on the microcirculation during free flap surgery when management was guided by detailed haemodynamic assessment.

In our animal experiment, anesthetized and mechanically ventilated pigs were randomised to receive Coll or Cryst infusion. Animals were bled until baseline SVI (Tbsl) dropped by 50% (T0), followed by resuscitation until initial SVI was reached (T4) in four steps. In our clinical trial patients undergoing maxillofacial tumour resection and free flap reconstruction were randomised into groups treated with either intra-operative Cryst or Coll solutions.

In the animal experiment, hemodynamic changes and GX degradation products serum levels followed similar pattern without significant difference between the groups. Animals received significantly less resuscitation fluid in the Coll as compared to the Cryst-group. In the clinical trial there was no difference between the groups regarding patient characteristics. Both groups remained haemodynamically stable throughout, but patients in the Cryst group required approximately 1.5 times more total fluid volume than in the Coll group. There was no significant difference in the microcirculatory blood flow between the groups as indicated by laser-Doppler flowmetry.

We concluded that: (1) volume-replacement ratio for solutions follows the Starling's principle when the GX is intact, and (2) when fluid management is guided by detailed haemodynamic assessment there was no difference between the effects of solutions on the microcirculation, hence it may not be the type of the fluid, but the appropriate VR caused haemodynamic stability and perfusion that is responsible for microcirculatory blood flow.

1. INTRODUCTION

Early fluid resuscitation remains the cornerstone of the treatment of severe hypovolemia, bleeding and septic shock. Although during these circumstances' fluid administration is a lifesaving intervention, it can also exert a number of adverse and potentially life-threatening effects, hence fluid therapy by-and-large is regarded a "double-edged sword".[1] Unfortunately, for the three fundamental questions of: "when", "what" and "how much", we don't have universally accepted answers. Nevertheless, not giving enough volume may result inadequate cardiac output (CO) and oxygen delivery (DO2), hence severe oxygen debt; while fluid overload can cause edema formation both in vital organs and on the periphery, hence impair tissue perfusion. Despite the broad acceptance of the importance of using appropriate parameters to guide our treatment during resuscitation, our current practice seems rather benevolent and uncoordinated worldwide as it was recently demonstrated in the FENICE trial.[2] In addition to using appropriate hemodynamic parameters to guide fluid resuscitation, the type of the infusion fluid should also be chosen carefully. Fundamentally Cryst or Coll are suitable for fluid resuscitation. Theoretically, Coll have better volume expansion effects, therefore they restore the circulating blood volume, hence DO2 faster than Cryst. However, the natural Coll albumin is very expansive, as compared to Cryst, but the cheaper synthetic Coll's or have several potential side effects. Ever since Coll's appeared on the scene the "crystalloid- colloid debate" has started, which seems like a never-ending story. At present this gigantic pendulum which swings our opinion between "good" and "bad" based on the actual evidence, points more to the latter when synthetic Coll's are concerned.

1.1 Starling's Hypothesis in the Context of the Glycocalyx

According to Starling's "3-compartment model" Cryst, with their sodium content similar to that of the serum, are distributed in the extracellular space, while Coll's should remain intravascularly due to their large molecular weight. Therefore, theoretically one unit of blood loss can be replaced by 3-4 units of Cryst and one unit of Coll solution.[3] This theory has a long history and due to early research data has broad acceptance worldwide since the 1960's.[4] However, several clinical trials including thousands of critically ill patients seem to disapprove this principle as we don't see this large difference in the required volume of Cryst versus Coll to stabilize these patients. Understanding physiology especially the role of the recently discovered multiple function of the endothelial GX layer

may cast a different light on these controversies. The purpose of this review is to highlight several issues, which should be taken into account when we are interpreting the results of recent clinical trials on Cryst and Coll fluid resuscitation. Starling's hypothesis revisited - in the context of the GX Fundamentally there are 3 infusion solutions that can be administer intravenously: 1) water, in the form of 5% dextrose, 2) Cryst's, containing sodium ions in similar concentration to that of the plasma, and 3) Coll's, which are macromolecules of either albumin or synthetic Coll molecules, such as hydroxyaethyl starches (HES), dextrans or gelatin solutions. According to the classic Starling view the main determinants of the fluid transport between the 3 main fluid compartments of the intracellular, interstitial and intravascular spaces, are determined mainly by the 2 semipermeable membranes: the endothelium and the cell membrane (Fig. 1). Water and glucose molecules can pass freely from the vasculature to the cells, hence they are distributed in the total body water. Sodium containing Cryst can pass the endothelium but not the cell membrane, hence these are distributed in the extracellular space, proportionally to the volume of the interstitial and intravascular compartments to the total extracellular fluid volume. Coll, because of their large molecular weight should remain intravascularly.[5]

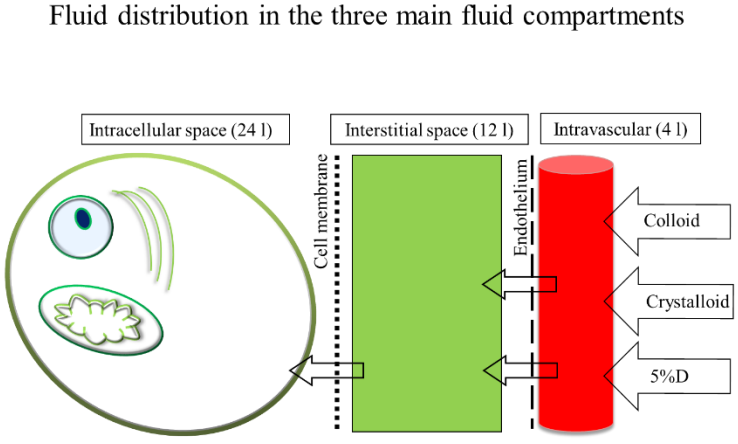

Figure 1.: Fluid distribution in the three main fluid compartments. In a normal (70kg) adult the total body water is about 60%, roughly 40 l divided into intracellular (~24l), interstitial (~12l) and intravascular (~4l) spaces separated by the endothelium and the cell membranes. According to the classic Starling "3 compartmental model" fluid distribution is mainly determined by these semipermeable membranes. Therefore, colloids should stay in the intravascular compartment, crystalloids are distributed in the extracellular space, and water, in the form of 5% dextrose (5D%) are distributed in the total body water. [5]

The filtration rate per unit area across the capillary wall are mainly determined by hydrostatic and Coll osmotic pressures as indicated by the classic Starling's equation:

$Jv = Kf ((Pc-Pi) - \sigma (\Pi i - \Pi c))$

Jv: Fluid movement, $(Pc-Pi) - \sigma (\pi i - \pi c)$: driving force, Pc: capillary hydrostatic pressure, Pi: interstitial hydrostatic pressure, Πi: interstitial oncotic pressure, Πc: capillary oncotic pressure, Kf: filtration coeficient, σ: reflection coeficient

However, there is some evidence that in most tissues the lymphatic flow would be insufficient to handle the extravasation of the amount of fluid as predicted by Starling, a phenomenon also termed as the "low lymph flow paradox".[6,7] It was proposed that it is the endothelial GX layer, which plays a pivotal role as a primary molecular filter and also provides an oncotic gradient, which was not included in the Starling's hypothesis.[8] A web of membrane-bound glycoproteins and proteoglycans on the luminal side of endothelium has been identified to form the GX layer. This compartment consists of many highly sulfated glycosaminoglycan chains providing negative charge for the endothelium. Due to these electrostatic properties the subglycocalyx space produces a Coll oncotic pressure that might be an important determinant of vascular permeability and thus fluid balance.[9] The structure and function of the endothelial GX varies substantially among different organ systems, and it is also affected by several inflammatory conditions.[10] In a recent experiment on isolated guinea pig heart, Jacob et al., observed a very interesting phenomenon.[11] They perfused the coronaries with Coll free buffer, isotonic saline, albumin and HES solution, and measured extravascular transudate and edema formation. The experiment was then repeated when the GX was stripped from the vessel wall by treating it with heparinase. With intact GX the net transudate, measured as hydraulic conductivity, was found to be 9.14 $\mu l\ min^{-1}\ g^{-1}$ tissue for Coll free perfusion, which was dramatically reduced when albumin was added in physiological concentration to the perfusate to 1.04 $\mu l\ min^{-1}\ g^{-1}$. It was also attenuated by HES supplementation but to a significantly lesser degree, to 2.67 $\mu l\ min^{-1}\ g^{-1}$. The founding that adding Coll's to the perfusate reduced extravasation, seemingly confirms the Starling's hypothesis, but interestingly, this effect did not correlate with the Coll osmotic pressure: albumin, which is a much smaller molecule than HES, had significantly better features in preventing transudate formation. This phenomenon is termed as the "colloid osmotic pressure paradox", which cannot be explained fully by the Starling's hypothesis and equation. One of the possible explanations is that the charges exposed by

molecules forming the GX are mainly negative, while albumin carries molecules like arginine and lysines with positive charges. There is some experimental evidence that these arginine groups are responsible for the effects of albumin on vascular permeability. On the contrary, HES molecules are uniformly negatively charged, which may explain the significant difference in hydraulic conductivity observed by Jacob and coworkers.[11]

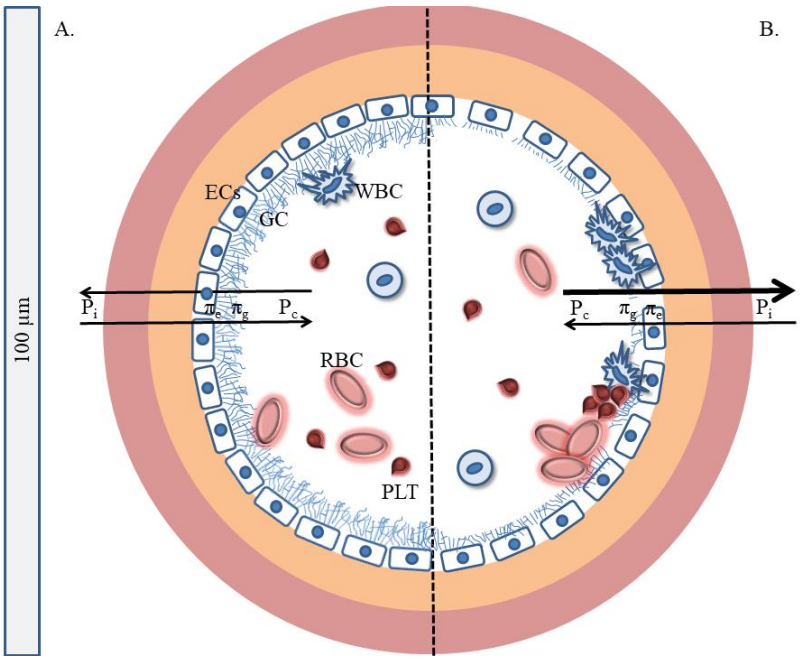

Figure 2.: Schematic transection of a capillary . A: In normal subjects the GX is intact and the Starling's concept is more-or-less valid and fluid transport is mainly determined by the Starling equation (see text). ECs, endothelial cells; RBC, red blood cells; PLT, platelets; WBC, white blood cells; Pi, interstitial hydrostatic pressure; πe, colloid osmotic pressures in the endtothelial surface layer; πg, colloid osmotic pressures directly below the endtothelial surface layer in the glycocalyx. B. In several critically ill conditions, both the glycocalyx and the endothelium becomes damaged. During these conditions the regulating functions of the endothelium and glycocalyx are partially or totally lost. These will affect fluid transport across the vessel walls with excessive fluid and protein extravasation, will cause leukocyte adherence and platelet adhesion, further impairing capillary blood flow, and the complex function of the endothelium and the microcirculation.[5]

They also suggested to modify the Starling equations as such:

$$Jv/A = Lp ((Pc - Pt) - (\Pi e - \Pi g))$$

Jv/A= filtration rate per unit area, Lp = hydraulic conductivity of the vessel wall, Pc−Pt = difference of hydrostatic pressure between the capillary lumen (c) and tissue (t), Πe = Coll osmotic pressures in the endtothelial surface layer, Πg = colloid osmotic pressures directly below the endtothelial surface layer in the GX

Nevertheless, under normal circumstances, when the GX is intact the Starling concept is still valid fluid transport is determined by the "Starling forces" (Fig. 2), and the volume replacement ratio should be several times higher for Coll's as compared to Cryst's. Indeed, several experimental models mainly on bleeding-resuscitation animal models reported the VR ratios for Coll as predicted by the Starling's hypothesis.[12–15] The explanation could be, that in animal models due to the relatively short experimental time, and because most models investigated hypovolemia, bleeding and resuscitation, the GX has no time for degradation. Nevertheless, these studies had different aims but to test the Starling's hypothesis, which should be performed in the future. GX has a pivotal role in not just to regulate endothelium permeability but it has an essential role in several others functions: it modulates shear force induced Nitric oxide synthesis and dismutation of oxygen free radicals in the endothelial cells; controls coagulation and inflammation by preventing platelet adhesion and leukocyte adherence to the vessel walls.[16] Therefore, it is not surprising that whenever the GX layer is damaged important pathophysiological changes take place, which can have serious effects on the function of the affected organ, or organs.

1.2. The Glycocalyx in the Critically Ill

There is mounting evidence that the GX becomes impaired or destroyed in several critically ill conditions. These include inflammation (both infectious or non-infectious), trauma, sepsis, ischemia-reperfusion injuries, but also persistent hypo-, and hyper-volemia are known to affect the GX.[17] During these circumstances the regulating functions of the endothelium and GX are lost, that can have serious effects on permeability hence fluid transport across the vessel walls with excessive fluid and protein extravasation (Fig. 2B), but other functions like leukocyte adherence and platelet adhesion are also affected. There is experimental evidence that during these circumstances the interstitial space becomes overwhelmed with Coll molecules.[11] Although albumin seemed to be somewhat more able to interact with these circumstances than HES but nevertheless it could not prevent Coll extravasation which was also enforced by increasing hydrostatic pressures. These experimental findings are in accord with the results of our former clinical study, in which

patients with septic shock and acute respiratory distress syndrome were administered either HES (molecular weight of 250 kD) or gelatin (30 kD) for treating hypovolemia. We applied detailed hemodynamic monitoring and did not observe any difference in the volume:replacing effects of these Coll, neither any change in the extravascular fluid volume, despite the huge difference in their molecular weight and Coll osmotic pressure.[18] This was possibly due to the very severe and long standing (meaning several days) condition of these patients, when it is highly likely that the GX was already severely damaged, hence "size" (i.e.: molecular weight) did not matter anymore. These observations have very important impact when we try to interpret the results of recent large clinical trials comparing Cryst and Coll in the critically ill. VR effects of Cryst and Coll in the critically ill.

1.3. Volume-replacement Effects of Crystalloids and Colloids in the Critically Ill

Although most recent large clinical trials were studies with end-points of 28-day mortality or organ dysfunction, it is worthwhile to analyze these results from a different perspective. One of the landmark trials is the SAFE study, published in 2004, where investigators compared the safety of albumin to normal saline in ICU patients (n= 6997). Results showed no significant difference between the groups in hemodynamic resuscitation end points, such as mean arterial pressure or heart rate, although the use of albumin was associated with a significant but clinically small increase in central venous pressure. The study showed no significant difference between albumin and normal saline regarding 28-day mortality rate or development of new organ failure.[19] SAFE was followed by the VISEP[20], CHEST[21] and 6S[22] trials all pointing more or less to a similar conclusion. Results showed strong association between acute kidney injury, increased use of renal replacement therapy and the use of hydroxyethyl starch solution, which was also accompanied with unfavorable patient outcome.[20–22] On the contrary, in the CRISTAL trial, which was designed to test mortality related to Coll and Cryst based fluid replacement in ICU patients, investigators detected a difference in death rate after 90 days, favoring the use of Coll. Furthermore, patients spent significantly fewer days on mechanical ventilation and needed shorter durations of vasopressor therapy in the Coll group than in the Cryst group.[23] There are several common features in these studies. First of all, the ratio of the administered volume of Cryst and Coll were completely different as what should have been expected according to the Starling principle. This is summarized in Table 1. In general, only 30-50% more Cryst seemed to have the same volume expanding effect as Coll. Based on these results, a

common view was formed that HES doesn't have higher potency for volume expansion than Cryst, but carries a greater risk of renal dysfunction and mortality.[19–26] However, it is important to note that none of these trials applied detailed hemodynamic monitoring, which is the second common feature of these studies. The administration of iv. fluids was mainly based on the clinicians' subjective decision[19,20,22,23,26], or on parameters such as heart rate[21], blood pressure[20,22,24], central venous pressure, urine output[19–22,24], lactate levels[21] or central venous oxygen saturation[20,22,24]. Cardiac output, stroke volume was not measured in most of the trials, which is essential to prove volume responsiveness, and it none of the applied indices detailed above are good monitoring tools of fluid-therapy.[1,27] Therefore, it is possible that considerable number of these patients were treated inappropriately. Although it is not the task of the current review, but it is important to note, that the methods on which fluid administration was indicated in these trials, also reflect our everyday practice, as it was nicely confirmed by a recent observational study.[2] In this large international survey it was revealed that fluid therapy is mainly guided by inadequate indices during our daily clinical routine. Therefore, one cannot exclude that in these trials, a considerable proportion of patients were not hypovolemic at all. Indeed, in the CHEST trial the mean values of the target parameters were as follows: heart rate of 89 min^{-1}, mean arterial pressure 74 mmHg, central venous pressure of 9 mmHg and serum lactate of 2 mmol l^{-1}.[21] None of these suggest hypovolemia, or at least it is highly unlikely that any of us would commence fluid resuscitation based on these values. There is some evidence that in healthy male subjects Coll solutions provided four times greater increase in blood volume as compared to saline, and extravasation was significantly higher after saline infusion.[28] Therefore, if we consider that a considerable proportion of these patients were critically ill, hence their GX was impaired, and despite they were not hypovolemic but received Coll, this may have led to an excessive extravasation. Furthermore, if fluid was administered to normovolemic patients, this could have caused increased hydrostatic pressures in the microcirculation leading to excessive HES extravasation and deposit of Coll molecules in the tissues, hence its side/toxic effects could be amplified further.

Table 1. Human randomised controlled trials				
Trial	**Population**	**Type of Fluids**	**Ratio of Cryst/ Coll**	**IH - Monit**
Finfer[19] (n=6933)	ICU patients	Albumin;Saline	1.32	No
Brunkhorst[20] (n=537)	Sever septic ICU patients	HES; RL	1.32	No
Myburgh[21] (n=7000)	ICU patients	HES 130/0.4; Saline	1.20	No
Guidet[24] (n=174)	Patients with severe sepsis	HES 130/0.4; Saline	1.23	No
Perner[22] (n=798)	ICU patients with severe sepsis	HES 130/0.42; Ringer's Acetate	1.00	No
Annane [23] (n=2857)	ICU patients with hypovolemic shock	Colloids (gelatins, dextrans, HES, 4% or 20% albumin); Crystalloids (isotonic or hypertonic saline, RL)	1.5	No
Yates [25] (n=202)	High risk surgical patients	HES 130/0.4; Hartman's solutions	1.69	No
Caironi[26] (n=1810)	Severe sepsis or septic shock	20% albumin ; Crystalloid	1.02	No
Lobo [28] (n=10)	healthy male subjects	Gelofusine or HES 6%; Saline	1.00	No

Table 1. Human randomised controlled trials. Cryst/Coll, ratio of crystalloid/colloid; HES, hydroxyethyl starch; ICU, Intensive Care Unit, RL: Ringer 's lactate, IH-Monit: invasive hemodynamic monitoring[5].

1.4. Haemodynamic management

Inappropriate haemodynamic management during major surgery may lead to hypoperfusion or fluid overload, both of which are accompanied by a significant risk of impaired postoperative outcome.[1] The same holds true for unnecessary use of vasoactive medications[29] and blood transfusions[30,31]. In reducing such adverse effects, advanced haemodynamic monitoring-based management has a strong pathophysiological rationale and, indeed, advanced haemodynamic monitoring-based peri-operative management has been shown to improve outcomes in high-risk surgery in several studies.[2,32] These trials showed that high-risk patients, especially those undergoing bowel surgery, benefited from

this approach, with reduced postoperative complications and increased survival. In addition to haemodynamic management, the type of fluid used may also have an important effect on outcome. Furthermore, the advantages of Coll solutions for the microcirculation have been shown in clinical studies applying goal-directed therapy, without inclusion of Cryst group as a control.[33,34] Therefore, one cannot exclude the possibility that the observed benefit of Coll on the microcirculation was due to better global haemodynamic conditions achieved by the better VR ratio of Coll (as compared with Cryst), and not due to their better microcirculatory properties per se.

Acute bleeding is a perilous condition requiring immediate intervention before hypoperfusion leads to severe organ damage and multiple organ dysfunction. In addition to the surgical control of bleeding, fluid resuscitation remains one of the most important life-saving interventions. The use of Coll and Cryst for resuscitation of bleeding patients has previously remained controversial with no definitive answer for the best course of action.[35–38] In trauma patients, hemorrhage has been proposed as the second most common contributing cause of death within 48 h following the injury.[39,40] In a multi-center analysis by Hoyt et al., hemorrhage was the primary cause of intraoperative death in 82% of patients with major trauma.[41] To avoid the lethal consequences of severe bleeding, intravenous fluid resuscitation is the first line of treatment, which has to be fast and efficient. Fundamentally, Cryst or Coll can be used for this purpose. Furthermore, as reported in recent prospective studies[42,43], non-survivor trauma patients also had significantly higher circulating syndecan-1 concentrations than survivors, indicating an impairment in the endothelial GX.[44–46] These results suggest that critical illness in general predisposes the patient to GX damage; hence, the VR ratio of Cryst and Coll may be different from what would have been expected.

2. AIMS AND SCOPES

I. The main aim of the current study was to compare the volume replacement effects of Cryst and Coll solutions during bleeding-resuscitation with moderate hemorrhage in an experimental animal model. We applied the model, which has been tested and reported in our previous experiments.[47,48]

II. Second, we aimed to perform a randomised clinical trial to examine the effects of intra-operative Cryst and Coll fluid replacement guided by detailed haemodynamic monitoring on microcirculatory perfusion in patients undergoing free flap surgery for maxillofacial malignancy.

3. METHODS

3.1.Volume-replacement ratio

3.1.1. Ethical permission

The experiments were performed on the EU Directive 2010/63/EU for the protection of animals used for experimental and other scientific purposes and carried out in strict adherence to the NIH guidelines for the use of experimental animals. The experimental project was approved by the National Scientific Ethical Committee on Animal Experimentation (National Competent Authority), Hungary, with license number: V./142/2013. The study was conducted in the research laboratory of the Institute of Surgical Research in a manner that did not inflict unnecessary pain or discomfort upon the animals.

3.1.2Animals and instrumentation

Vietnamese pot-bellied pigs (n=30) underwent a 12-h preoperative fasting period with free access to water. The pigs were randomised into two groups: balanced crystalloid Ringerfundin, RF group (B. Braun AG) and a colloid (Voluven®, hydroxyethyl starch (HES)) group. Anesthesia was induced by intramuscular injection of a mixture of ketamine (20 $mgkg^{-1}$) and xylazine (2 $mgkg^{-1}$), maintained by a continuous intravenous propofol infusion (6 $mgkg^{-1}h^{-1}$ i.v.), and analgesia was performed with nalbuphine (0.1 $mgkg^{-1}$). Tracheal tubes were inserted in all animals, and the lungs were mechanically ventilated by Dräger Evita XL (Dräger, Lübeck, Germany). Tidal volume was adjusted to 10 $mlkg^{-1}$, and the respiratory rate was initialized to keep the end-tidal carbon dioxide and partial pressure of arterial carbon dioxide within physiological range (35–45 mmHg). The adequacy of anesthesia was assessed by checking jaw stiffness. After induction of anesthesia, catheters were inserted into the right jugular vein, the left carotid artery, and the right femoral artery via aseptic dissection of the vessels. For invasive hemodynamic monitoring, a transpulmonary thermodilution catheter (PiCCO, PULSION Medical Systems SE, Munich, Germany) was placed in the right femoral artery (3 mm). A central venous catheter was implanted into the right jugular vein and was positioned by the guidance of intracavital ECG. Throughout bleeding, blood was drained through a catheter from the left carotid artery to a cylinder. An external warming device was used to retain the animals' body temperature at 37±1 °C.

3.1.3Experimental protocol

The study protocol is summarized in Fig. 3. Briefly, after instrumentation, 30 min was allowed for stabilization before baseline (Tbsl) measurements were taken. At each assessment point, hemodynamic measurements, blood gas analyses, and laboratory tests were performed. After Tbsl, the pigs were bled until the stroke volume index dropped to 50% of its baseline value (T0); then, measurements were repeated. The difference of stroke volume index (SVI) at Tbsl and T0 was divided into four equal target values, which was planned to be reached in four steps during fluid resuscitation (T1–4) to reach the initial SVI by T4. Fluid replacement was executed with boluses of balanced RF or HES solutions until the target SVI value was reached. After reaching each step, 20 min was allowed for equilibrium; then, blood gas and hemodynamic parameters were measured. All of the pigs were euthanized with sodium pentobarbital at the end of the experiment.

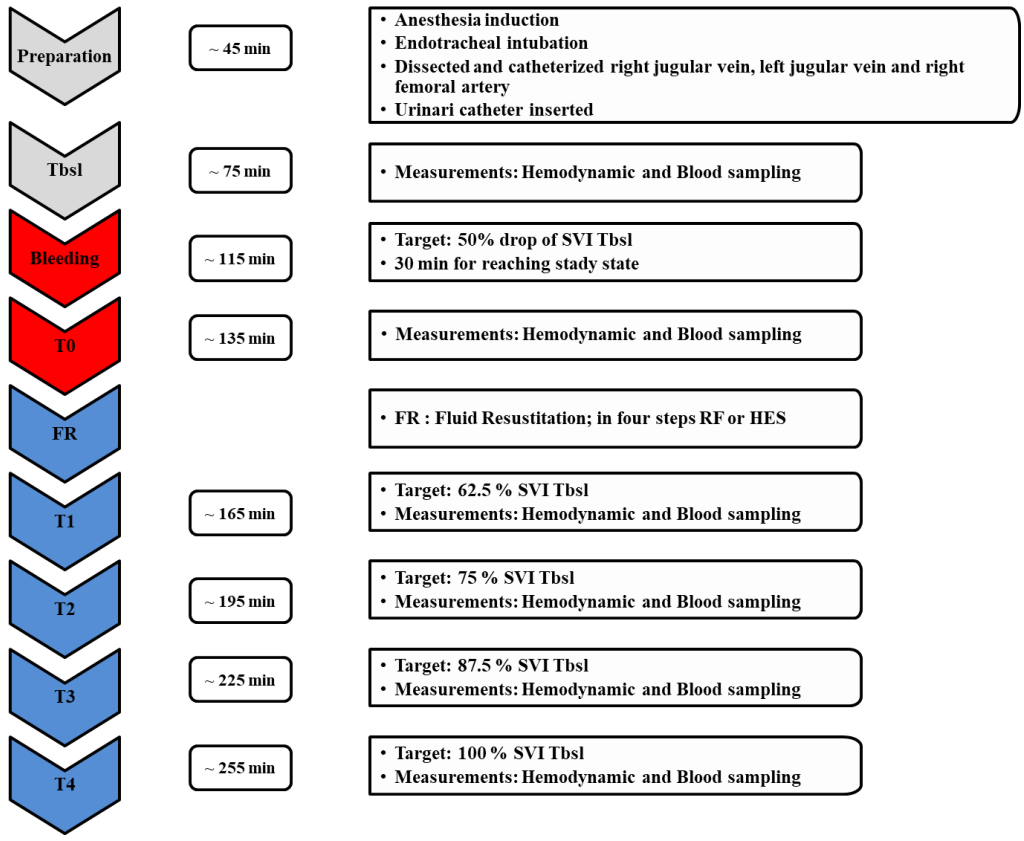

Figure 3. Schematic flowchart illustrating the experimental protocol. After baseline measurements, animals were bled until the stroke volume index (SVI) decreased by 50% (T0). Then, measurements were repeated and the animals were randomised into the balanced crystalloid (Ringerfundin®, RF B. Braun AG) or colloid (Voluven®, HES) groups. The difference of the SVI *Tbsl* −SVI *T0* was divided into four equal steps (*T1–4*) and i.v. fluids were administered to reach these target values.

3.1.4Hemodynamic monitoring and blood gas sampling

Cardiac function (CFI), cardiac index (CI), left ventricular contractility (dPmax), global end-diastolic volume (GEDI), heart rate (HR), mean arterial pressure (MAP), pulse pressure variation (PPV), stroke volume index (SVI), and stroke volume variation (SVV) were measured via transpulmonary thermodilution and pulse contour analysis at baseline and at the end of each step. All hemodynamic parameters were indexed for body surface area or body weight. Ten milliliters of less than 8 °C cold isotonic saline was injected through the jugular catheter for thermodilution-based measurements, and the average of three boluses recorded at the end of each interval. Central venous pressure (CVP) was measured via the jugular catheter in parallel with the other hemodynamic parameters. For blood gas measurements, the right femoral artery served as the site for arterial blood gas sampling and the catheter in the internal jugular vein was used for taking central venous blood gas samples. These were analyzed in parallel by co-oximetry (Cobas b 221, Roche Ltd., Basel, Switzerland) at baseline and at the end of each resuscitation step. From these parameters, the following variables were calculated.[49]

Oxygen consumption (VO_2): $VO_2 = CI \times (CaO_2 - (Hgb \times 1.34 \times ScvO_2 (0{:}003 \times PcvO_2)))$

Oxygen delivery (DO_2): $DO_2 = CI \times (Hgb \times 1.34 \times SaO_2 + 0.003 \times PaO_2)$

Oxygen extraction = VO_2 / DO_2

VR ratios were calculated by the resuscitation fluid over the total blood loss.

3.1.5 Glycocalyx degradation

Blood concentrations of syndecan-1 and glypican were quantified by enzyme-linked immunosorbent assay (ELISA) (MybioSource, Inc., San Diego, USA). For this purpose, blood samples were taken at Tbsl, T0, and T4; then, the blood was centrifuged and the serum stored at −80 °C.

3.2 Effects of goal-directed crystalloid vs. colloid fluid therapy on microcirculation during free flap surgery

3.2.1 Patient selection

This randomised, controlled study (Ethical Committee No. 44/2014) was undertaken between April 2014 and February 2018 and was approved by the Regional and Institutional

Human Medical Biological Research Ethics Committee, University of Szeged, Hungary on 28 April 2014. The investigation was performed at the University of Szeged. The study was registered at ClinicalTrials.gov with the registration number: NCT03288051. Written informed consent was obtained from all participants. Adult patients of both sexes undergoing radical forearm free flap surgery were recruited. Exclusion criteria included vulnerable individuals as defined in ISO 14155:2011, pregnant or lactating women, and end-stage oral cancer. The progress of participants through the study is depicted in Fig. 4. The study's primary end-point was the difference in the perfusion units as determined by laser-Doppler at R_{12}. On the basis of preliminary results, for a study to have 80% power to show a significant difference in perfusion units at R_{12}, when the standardised difference (clinically significant difference/standard deviation) worked out to be 0.9, required a minimum of 30 patients in total (15 per group).

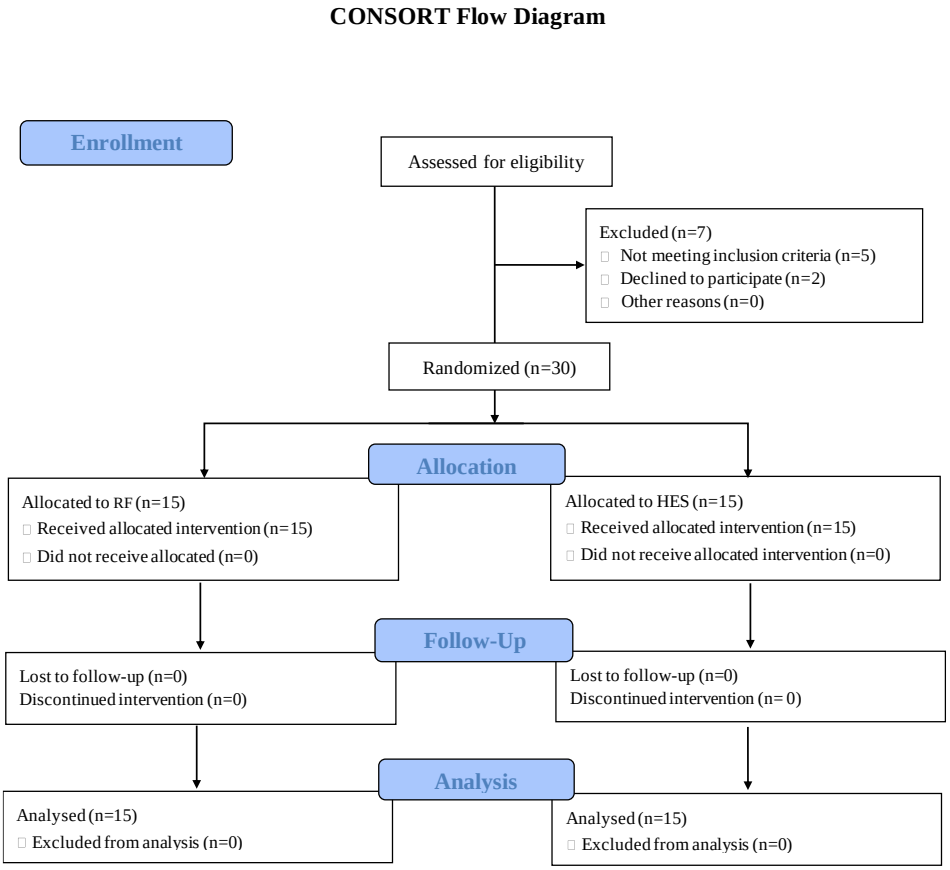

Figure 4.: Flow chart according to the CONSORT (Consolidated Standards of Reporting Trials) statement showing the progress of participants throughout the study.

Patients were randomised either to a Cryst group (Ringerfundin; B. Braun Melsungen, Germany) or a Coll group [hydroxyethyl starch (HES), Voluven 6%; Fresenius Kabi Deutschland, Germany], using envelope block-randomisation in blocks of fifteen. Patient enrolment, sequence generation and assignment to interventions were performed by a responsible investigator. Only the patients were blind to group allocation.

3.2.2 Intra-operative protocol

Patients received routine anaesthetic management. In addition to standard monitoring, a radial artery catheter was inserted under local anaesthesia for invasive blood pressure monitoring. This arterial line was also connected to a noncalibrated haemodynamic monitor (ProAQT; PULSION Medical Systems SE, Munich, Germany). Anaesthesia was induced with 1 to 3 mg kg^{-1} propofol [Propofol (1%), Fresenius Kabi Deutschland, Germany], 0.6 mg kg^{-1} rocuronium (Esmeron, MSD Pharma Hungary,Hungary),and for analgesia, morphine (Morfina Jacopo Monico, Italy) was used. Anaesthesia was maintained with sevoflurane with the minimum alveolar concentration maintained around 1.3 vol%. Patients were ventilated with an 8 ml kg^{-1} tidal volume, in pressure control mode, in order to have a reasonable effect on pulse pressure variation (PPV).[32,50] During the operation, core temperature was measured by rectal thermometer. Maintenance fluid was Ringerfundin 1ml kg^{-1} h^{-1}.

After induction of anaesthesia, a central venous catheter was inserted into the right internal jugular or the right subclavian vein based on the requirements of the surgical approach. Haemodynamic assessment during the operation was based on a multimodal concept shown in Fig. 5. This included elements of the model that had been applied and reported in a recent multicentre clinical trial, in which our institute also participated.[2]

In brief, fluid responsiveness was defined as PPV at least 10%, but this this did not mean that fluid was given immediately: fluid administration was determined by a complex, multimodal algorithm, depicted in Fig. 5. In cases when fluid loading was indicated, a bolus of 250ml of Ringerfundin or HES, as determined by randomisation, was administered within 15min. The aim was to maintain cardiac index (CI) above 2.5 l min^{-1} m^{-2}. If CI was low, and our haemodynamic model indicated that contractility had to be improved, then dobutamine (Dobutamine Hexal, Sandoz Hungaria, Hungary) was administered starting at a rate of 5 µg kg^{-1} min^{-1}. In the case of a drop in blood pressure, as indicated by a mean

arterial pressure of 65 mmHg or less or at least 20% drop as compared with baseline data, after excluding hypovolemia, or myocardial depression, norepinephrine was started as a continuous infusion.

Global haemodynamic assessment was complemented with measuring hourly urine output and arterial and central venous blood gas analysis. Parameters included in the haemodynamic decision algorithm were central venous oxygen saturation ($ScvO_2$), central venous-to arterial CO_2-gap (dCO_2), arterial lactate, HCO_3 and pH. Normal values for these parameters were considered as $ScvO_2$: 70 to 80%, dCO_2 of 6 mmHg or less, HCO_3: 20 to 24 mmol l^{-1}, pH: 7.35 to 7.45. Arterial and central venous samples were taken at the same time hourly, or anytime in between, when a decision had to be supported in order to commence therapy. This approach was aimed at helping to individualise treatment, rather than following a preset target value.

Data were recorded after instrumentation at baseline (T_0), at incision (T_i) and then hourly until the end of the surgery (T_{es}) and 24h after T_0 (T_{24}).

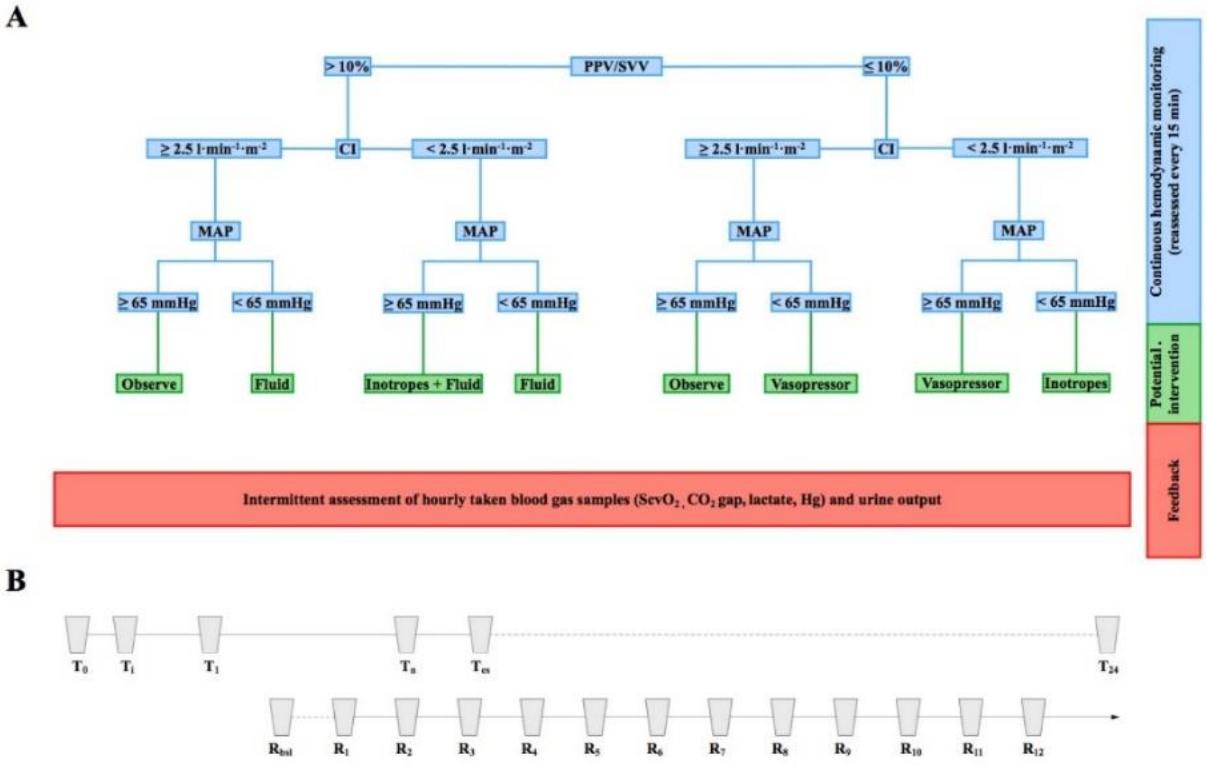
A
PPV/SVV
> 10%
≤ 10%
≥ 2.5 l·min⁻¹·m⁻²
CI
< 2.5 l·min⁻¹·m⁻²
MAP
≥ 65 mmHg
< 65 mmHg
Observe
Fluid
Inotropes + Fluid
Vasopressor
Inotropes
Continuous hemodynamic monitoring (reassessed every 15 min)
Potential intervention
Feedback
Intermittent assessment of hourly taken blood gas samples ($ScvO_2$, CO_2 gap, lactate, Hg) and urine output
B
T_0
T_i
T_1
T_n
T_{es}
T_{24}
R_{bsl}
R_1
R_2
R_3
R_4
R_5
R_6
R_7
R_8
R_9
R_{10}
R_{11}
R_{12}

Figure: 5. (A) Haemodynamic assessment and interventions. CI, cardiac index; Hgb, haemoglobin; MAP, mean arterial pressure; PCO_2 gap, central venous-to-arterial PCO_2-gap; PPV, pulse pressure variability; $ScvO_2$, central venous oxygen saturation; SVV, stroke volume variation. (B) Measurements and recordings: T_0, baseline; T_i, incision; T_{es}, end of surgery; T_{24}, day one. R_{bsl}, baseline (Laser-Doppler after flap was prepared); R_1, one hour after $R_{2–12}$, hourly.

3.2.3 Laser-Doppler flowmetry

All flaps were monitored with noninvasive laser-Doppler flowmetry (PeriFlux 5000 LDPM; Perimed, Järfälla, Sweden) intra-operatively, and postoperatively. A probe with a standard fibre separation of 0.25mm, and a 780nm wavelength laser was used. The depth of the measurements was 0.5 to 1mm. Results are expressed as perfusion units. The first measurements were taken after the flap was prepared (R_{bsl}), then 1h after reperfusion and continued hourly for up to 12h (R_1–R_{12}). The probe was placed and fixed in a position in the centre of the forearm flap skin island. The skin in the deltoid region provided the control site. At both places, measurements were taken after active warming of the skin, at 35℃ and 44℃. Data were recorded for more than 2min at each measurement point. Quantitative assessment of the recording periods was performed off-line.

3.2.4 Postoperative and ICU protocol

All patients were monitored in the ICU until they were discharged to the Maxillofacial Surgery ward. Patients received standard ICU care according to our institutional protocols.

3.3. Data analysis and statistics

For statistical analysis, Statistical Program for Social Sciences version 23.0 for Windows (SPSS, Chicago, Illinois, USA) was used, and *P* value less than 0.05 was considered as significant. Data are presented as mean ± SD or median [IQR]. For testing normal distribution, the Shapiro–Wilk test was used. Independent samples were tested by independent samples t-test or Mann–Whitney U test, as appropriate. Changes in repeated measures throughout the experiment were tested by two-way repeated measures analysis of variance (ANOVA) with Bonferroni post hoc comparisons. Categorical data were compared using χ^2 tests. The Type I error probability associated with this test of the null hypothesis is 0.05.

4. RESULTS

4.1. Volume-replacement ratio

Out of the 30 animals, 27 survived the full experiment. Two in the HES group and one in the RF group had a sudden cardiac arrest after induction of anesthesia for reasons unknown. Therefore, the results of 27 animals (HES n = 13; RF n = 14) were finally analyzed. Demographics and overall data on fluid management are summarized in Table 2. Animals were of similar weight, height, and body surface area in both groups. For a 50% decrease in SVI, a similar amount of blood had to be drained in both groups. Invasive hemodynamic (PiCCO) measurements were taken at similar frequencies in both groups. Urine output was significantly higher in the RF group.

Table 2. Demographics, blood loss and fluid therapy

	HES (n=13)	RF (n=14)	P
Weight (kg)	26.0 [22.5-28.0]	25.5 [24.0-37.0]	0.280
Height (cm)	118.0 [112.5-120.0]	115.0 [110.0-125.0]	0.981
BSA (m^2)	0.91 [0.855-0.97]	0.94 [0.975-1.115]	0.401
Shed blood (ml)	505.6 ± 159.3	469.7 ± 127.3	0.529
Total blood loss (ml m^{-2})	552.8 ± 174.9	481.1 ± 95.2	0.197
PiCCO measurements (n)	23.0 ± 8.0	25.0 ± 5.0	0.422
Saline used for PiCCO measurements (ml)	230.0 ± 81.5	252.1 ± 58.2	0.422
Urine (ml)	450 [350-626]#	759.5 [421-1110]	<0.001

Table 2. Data are presented as mean ± standard deviation or median [IQR], # $P < 0.05$ significantly different between groups.

4.1.1. Macro-hemodynamic effects of fluid resuscitation

Hemodynamic results were similar at Tbsl, and goals of 50% reduction in SVI were reached by T0 in both groups (Table 3). Hemodynamic changes during the experiment did not show clinically relevant differences between the groups. At Tbsl, the SVI values were similar, after bleeding SVI decreased by the planned 50% to T0 and returned to its initial value by

T4. Kinetics of the CI, MAP, HR, and GEDI showed similar pattern in both groups with significantly higher values in the HES group at the end of the experiment (T4). SVV and PPV almost doubled after bleeding in both groups and then returned to baseline values, being significantly lower in the HES group. Extravascular lung water index showed some changes during the experiment in both groups, without any significant differences between the groups. Contractility, as indicated by dPmax values, also showed same changes in both groups.

Table 3. Hemodynamic parameters during hemorrhage and fluid resuscitation

	Group	Tbsl	T0	T1	T2	T3	T4
Stroke volume index (ml m^{-2})	HES	34.4 ± 7.5†	16.5 ± 3.6*	22± 4.7*†	25.8 ± 5.2*†	30.3 ± 5.7*†	34.3 ± 7.2†
	RF	33 ± 3.6†	15.4 ± 2.2*	19.6 ± 3.8*†	23.9 ± 4.0*†	28.0 ± 5.5*†	32.3 ± 3.3†
Cardiac index (l min^{-1} m^{-2})	HES	3.25 ± 0.23†	1.58 ±0.27*	2.38 ±0.27*†	2.75 ±0.29*†	3.36 ±0.34#†	3.99 ±0.54#†
	RF	3.14 ± 0.19†	1.84 ±0.4*	2.22 ±0.43*†	2.52 ±0.34*†	2.90 ±0.29*†	3.39 ±0.36†
Mean arterial pressure (mmHg)	HES	122± 15.2†	82± 25.9*	110± 27.5#†	114± 24.5#†	121± 25.2#†	123± 23.7#†
	RF	124± 16.6†	69± 17.1*	77± 18.3*†	90± 15.4*†	99± 18.0*†	101± 9.9*†
Heart rate (min^{-1})	HES	95± 18.5	105± 27.5	106± 24.3*	106± 24.2*	109± 20.8*	117± 16.7#*†
	RF	97± 18.4†	111± 19.6*	107± 16.2*†	106± 18.7*†	102± 15.1†	102± 13.8†
Global end-diastolic volume (ml m^{-2})	HES	361 ± 60.6†	222 ± 36.4*	267 ± 45.2*†	283 ± 46.2*†	333 ± 54.2#†	351 ± 55.5#†
	RF	329 ± 46.8†	212 ± 52.5*	231± 51.6*†	249 ± 40.8*†	280 ± 47.3*†	300 ± 42.9*†
Stroke volume variation (%)	HES	11.4 ± 5.9†	23± 7.0*	18.1 ± 6.8*†	13.8 ± 3.2†	11.7 ± 4.7†	6.7 ± 2.7#*†
	RF	11.7 ± 3.0†	21.8 ± 6.0*	19.3 ± 5.4*	16.3 ± 4.4*†	13.8 ± 4.4†	10.3 ± 2.5†
Pulse pressure variation (%)	HES	10.3± 3.0†	24.2± 6.0*	16.6± 3.7#*†	13.1± 3.5†	9.8± 2.0†	6.9± 2.3#*†
	RF	10.5± 4.8†	24.4± 5.4*	22.1± 6.2*	16.8± 5.7*†	13.8± 5.6*†	10.3± 2.4†
Systemic vascular resistance index (dyn × scm^{-5} m^{-2})	HES	2937± 359	3517 ± 1094	3618 ± 831#*	3183 ± 650	2796 ± 483†	2309 ± 277*†
	RF	3057± 510	2919 ± 545	2664 ± 570	2793 ± 628	2632 ± 527*	2345 ± 433*†
EVLWI (ml kg^{-1})	HES	11.22± 5.7†	10.88± 7.0*	11.66± 6.6*	12.00± 6.1*	12.22± 7.1	12.66± 7.0†
	RF	9.61± 2.1	9.07± 2.3	8.76± 1.2*	8.76± 0.9	8.84± 1.0	9.46± 1.6
dPmax (mmHg s^{-1})	HES	703 ± 187.5†	612± 118.2*	717± 121.6†	771± 125.3†	791± 147.6†	811± 144.9*†
	RF	588± 246.8	588± 286.3	642± 233.0†	611± 278.3	639± 229.3	671± 223.8*†

Table 3.: Data are presented as mean ± standard deviation or median [IQR], * $P < 0.05$ significantly different between Tbsl , † $P < 0.05$ significantly different between T0 , # $P < 0.05$ significantly different between groups.

4.1.2. Changes in VO2/DO2 during fluid resuscitation

Blood gas parameters during hemorrhage and fluid resuscitation are summarized in Table 4. Arterial pH was elevated in both groups due to unintentional hyperventilation which was then corrected towards the end of the experiment. Partial pressure of arterial oxygen tension and oxygen saturation remained stable and within the normal range throughout the study. Central venous oxygen saturation fell during the bleeding phase in both groups, but baseline values were achieved earlier in the HES group. Changes in oxygen extraction followed a similar pattern in both groups. Venous to arterial carbon dioxide gap increased significantly after the bleeding phase, with significantly higher values in the RF group, and then returned to physiological values by T3 in both groups.

Table 4. Blood gas parameters during hemorrhage and fluid resuscitation

	Group	Tbsl	T0	T1	T2	T3	T4
a pH	HES	7.613± 0.038$^{\#}$	7.603± 0.061$^{\#}$	7.568± 0.068$^{\#}$	7.54± 0.052	7.513± 0.043*†	7.519± 0.042*†
	RF	7.541± 0.049	7.498± 0.063	7.448± 0.055*†	7.393± 0.161*	7.464± 0.055*	7.48± 0.061*
$PaCO_2$ (mmHg)	HES	28.1 ± 3.2	24.3 ± 3.8$^{\#}$	26.1± 4.6$^{\#}$	28.4± 4.4$^{\#\dagger}$	29.9± 3.1$^{\#\dagger}$	30.8± 4.5†
	RF	33.8 ± 5.3	33.9 ± 7.4	39.1 ± 6.8†	38.6 ± 6.8†	39.4 ± 6.6†	38.3 ± 6.0†
PaO_2 (mmHg)	HES	87.5 ± 12.2†	102.8 ± 11.0*	100.4 ± 9.6*	95.8± 8.7	90.3± 13.0†	84.5± 14.6†
	RF	99.0± 24.7	102.0± 25.7	96.7± 27.8†	99.2± 29.3	94.9± 27.5	99.6± 30.9
HCO^{-}_3 (mmol l^{-1})	HES	27.6± 2.3†	24.4± 1.8*	23.0± 1.5$^{\#}$*	23.1± 2.0*	23.5± 1.2$^{\#}$*	24.4± 1.7$^{\#}$*
	RF	28.1± 1.4	25.5± 4.0	26.3± 3.3	26.6± 3.6	27.5± 2.7†	27.7± 2.5†
SaO_2 (%)	HES	97.8± 0.4	98.4± 0.3	98.2± 0.3	98± 0.4	97.7± 0.5	97.7± 0.4
	RF	97.8± 1.2	97.8± 1.1	96.5± 2.7†	96.9± 2.4	96.8± 2.2†	96.8± 2.6
a Lactate (mmol l^{-1})	HES	3.8± 2.0†	6.9± 3.7*	8.1± 3.0$^{\#}$*†	7.6± 2.2$^{\#}$*	7.1± 2.0$^{\#}$*	5.5± 1.9$^{\#}$*†
	RF	2.1± 0.8†	4.3± 2.0*	4.7± 2.0*	4.2± 1.8*	3.8± 1.7*	3.3± 1.4*
Hct (%)	HES	30.0± 4.8	26.7± 5.3	23.8± 5.2*†	22.5± 4.5*†	20.6± 3.5*†	19.3± 3.9*†
	RF	34.5± 4.6†	30.5± 5.6*	27.1± 4.5*†	25.1± 3.9*†	23± 2.6*†	22.4± 3.1*†
a Hb (g dl^{-1})	HES	9.8± 1.2	8.9± 1.4	8.1± 1.5*†	7.7± 1.3*†	7.1± 0.9*†	6.7± 1.0*†
	RF	10.9± 1.2	9.9± 1.6	9± 1.3*†	8.4± 1.1*†	7.7± 0.9*†	7.7± 1.0*†

Table 4. Data are presented as mean ± standard deviation or median [IQR], * $P < 0.05$ significantly different between Tbsl , † $P < 0.05$ significantly different between T0 , # $P < 0.05$ significantly different between groups

Table 4. Blood gas parameters during hemorrhage and fluid resuscitation *(Continued)*

	Group	Tbsl	T0	T1	T2	T3	T4
$PcvCO_2$ (mmHg)	HES	31.4± 2.7$^{\#}$	30.3± 4.7$^{\#}$	30.8± 5.3$^{\#}$	31.7± 3.6$^{\#}$	33.2± 3.7$^{\#}$	33± 3.7$^{\#}$
	RF	39.4± 7.6	43.2± 7.6	47.6± 6.9*†	46.1± 7.1	44.3± 7.5	42.1± 6.6
$PcvO_2$ (mmHg)	HES	46.7± 8.1†	36.0± 9.9*	40.7± 7.8*†	44.5± 7.8†	46.8± 5.2†	45.4± 8.7†
	RF	48.3± 7.2†	34.9± 4.0*	39.3± 4.1*†	44.8± 3.3†	46.2± 5.2†	48.6± 6.7†
$ScvO_2$ (%)	HES	83.9± 7.3†	69.1± 14.2*	76.0± 9.9*	79.7± 5.9*†	82.6± 4.2†	82.8± 4.8†
	RF	83.5± 8.1†	59.9± 6.6*	65.7± 7.9*	72.7± 7.4*†	76.2± 6.4*†	79.0± 8.1*†
Oxygen delivery index (ml min^{-1} m^{-2})	HES	58.6± 26.8	58.7± 27.6	62.3± 34.7	55.0± 21.8	49.7± 16.2	52.9± 20.5
	RF	65.2± 32.9†	97.5± 36.9*	85.3± 22.5*	69.4± 16.5†	63.4± 23.5†	65.5± 31.0†
Oxygen consumption index (ml min^{-1} m^{-2})	HES	58.6± 26.8	58.7± 27.6	62.3± 34.7	55.0± 21.8	49.7± 16.2	52.9± 20.5
	RF	65.2± 32.9†	97.5± 36.9*	85.3± 22.5*	69.4± 16.5†	63.4± 23.5†	65.5± 31.0†
Oxygen extraction (%)	HES	14.2± 7.2†	29.8± 14.2*	22.6± 9.9*	18.6± 6.0†	15.5± 4.5†	15.2± 5.1†
	RF	14.5± 8.2†	38.8± 6.8*	32.0± 7.1*	25.0± 6.2*†	21.3± 6.5*†	18.4± 8.3*†
Venous to arterial carbon dioxide gap (mmHg)	HES	3.3± 1.4†	6.0± 2.8$^{\#}$*	4.7± 2.0$^{\#}$	3.3± 1.9$^{\#\dagger}$	3.3± 1.2†	2.2± 1.2†
	RF	5.6± 3.4†	9.3± 1.6*	8.5± 1.7*†	7.5± 2.6	4.9± 2.0†	3.8± 2.2*†

4.1.3. Volume-replacement ratios

While the hemodynamic profile was very similar, there were significant differences between the groups in the total amount of fluid required and in the ratio of the resuscitation fluid over the total blood loss. Significantly more RF was used during resuscitation than HES (Fig. 6). Calculating the VR ratio, it was significantly higher in the RF group, where almost three times more RF was required to achieve the same hemodynamic parameters (Fig. 7.)

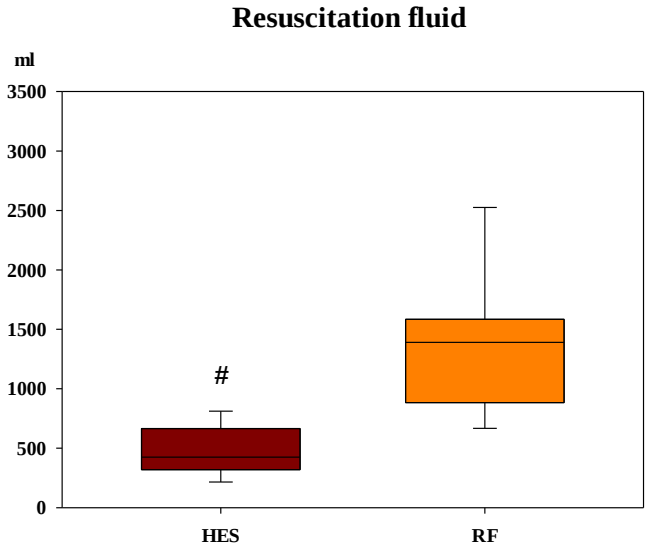

Figure 6: Resuscitation fluid (milliliters). Data are presented as median [IQR]. *P* = 0.002

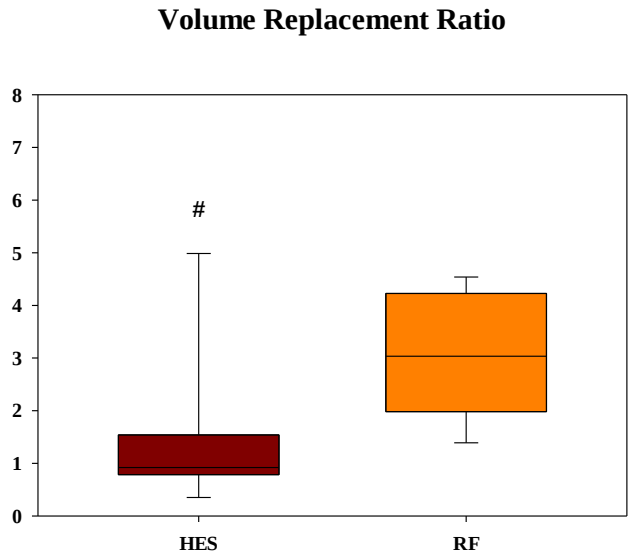

Figure 7: VR ratio: resuscitation fluid/total blood loss. Data are presented as median [IQR]. *P* = 0.002

4.1.4. Endothelial function

Plasma concentration of syndecan-1 was significantly lower in the RF group at T0 and T4 between Tbsl values (Fig. 8a). Values of glypican in the RF group were significantly lower at T4 compared to Tbsl and T0 (Fig. 8b). However, the syndecan-1 hematocrit ratio and the glypican hematocrit ratio showed no significant differences throughout the whole experiment (Fig. 8c–d).

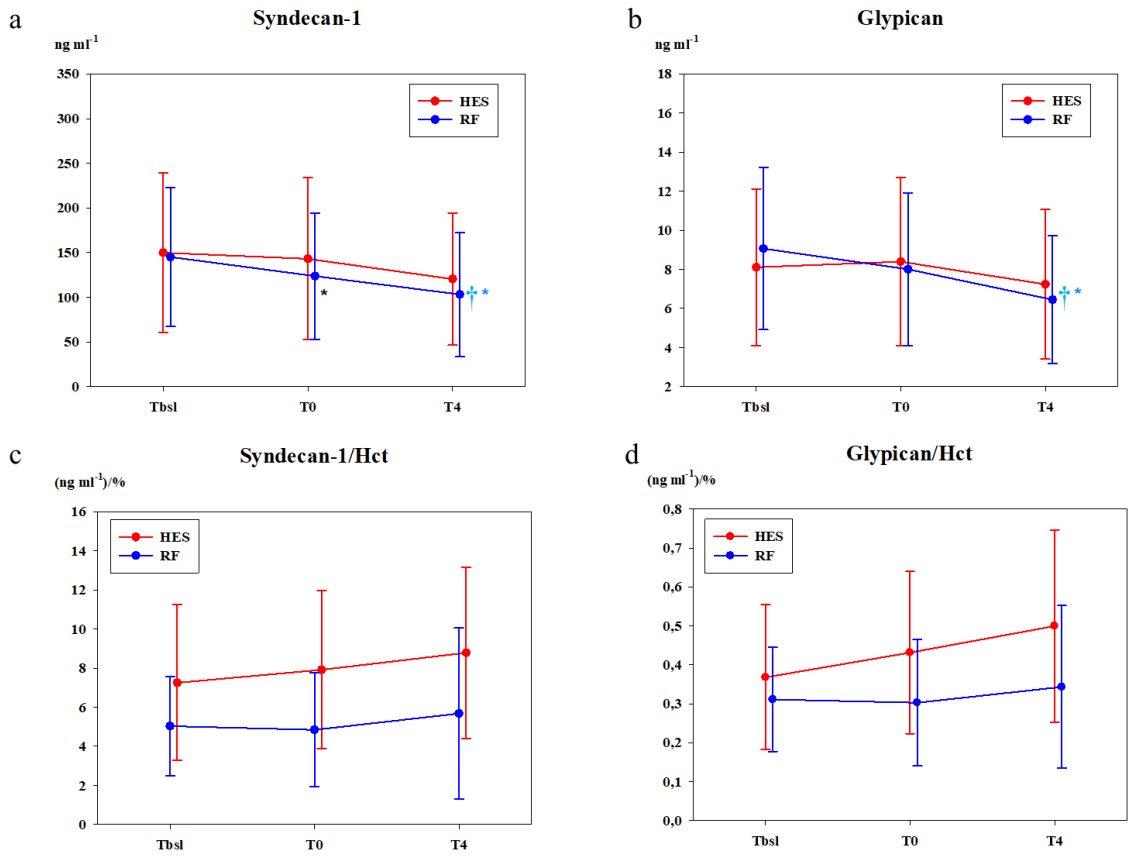

Figure 8: Endothelial function. Plasma concentrations of syndecan-1 (a), glypican (b), syndecan-1 hematocrit ratio (c) and the glypican hematocrit ratio (d) are delineated. Data are presented as mean ± standard deviation. HES = colloid group, RF = crystalloid group

4.2. Effects of goal-directed crystalloid vs. colloid fluid therapy on microcirculation during free flap surgery

There was no difference in the demographics, as summarised in Table 5. Complete flap failure occurred on five occasions (one in the Ringerfundin, four in the HES group, *P* = 0.142).

Table 5. Demographic variables of patients in the crystalloid (Ringerfundin, RF)- and colloid (hydroxyethyl starch, HES)-treated groups.

	RF (*n*=15)	HES (*n*=15)	(*P*=)
Female/ Male	5/10	3/12	0.409
Age (year)	64±11	62±10	0.680
Height (cm)	170.6±9.0	168.8±7.2	0.550
Weight (kg)	72.0[60.0-83.0]	62.0[57.0-81.0]	0.539
BMI (kg m^{-2})	24.4[21.6-27.8]	24.0[19.6-31.6]	0.775
Scores			
APACHE II (point)	13.5[9.5-15.25]	14.0[7.75-16.0]	0.946
APACHE MR (%)	17.6±6.8	16.7±8.3	0.731
ASA 1 (n)	3	0	
ASA 2 (n)	9	10	0.250
ASA 3 (n)	3	5	
Procedures			
Duration of operation (min)	354.3±59.4	342.4±79.1	0.644
Duration of ischemia (min)	66.9±13.1	67.9±19.3	0.869
LOS			
ICU (day)	2[2-3]	2[2-2]	0.389
Hospital (day)	9[7-13]	10[8-15]	0.325
Organ support on ICU			
Mechanical ventilation (day)	1[1-1]	1[0-1]	0.595
Vasopressor (day)	0[0-1]	1[0-1]	0.683
Dialysis (therapy)	1	0	

Table 5. Data are presented as mean ± standard deviation or median [IQR]. BMI = body mass index, APACHE II = Acute Physiology and Chronic Health Evaluation II, APACHE MR = Acute Physiology and Chronic Health Evaluation Mortality Rate, ASA = American Society of Anaesthesiologists' classification, LOS= Length of Stay, ICU= Intensive Care Unit.

4.2.1. Macrohaemodynamic effects of fluid resuscitation

Patients remained haemodynamically stable throughout the observation period in both groups (Table 5.). PPV was in general higher in the Ringerfundin group, which became significant at T_3. CI also showed the same pattern in both groups, with significantly higher values in HES group at T_1. At T0, the systemic vascular resistance index values were significantly elevated in the Ringerfundin group. Several other parameters showed changes during the experiment, without any significant differences between the groups. Intra-operative total urine output reached similar values in the HES and Ringerfundin-treated groups [355.0 (166.4) ml and 477.3 (212.5) ml, respectively, $P = 0.090$]. Creatinine values as measured 24 h after surgery were not different between the groups either [HES, 76 (19) mmol l^{-1} ; Ringerfundin, 71 (26) mmol l^{-1} , $P= 0.505$].

Table 6. Hemodynamic parameters in the crystalloid (Ringerfundin, RF)- and colloid (hydroxyethyl starch, HES)-treated groups during the study period.

	Group	T_0	T_i	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_{es}	T_{24}
SAP (mmHg)	RF	115.1±48.1	119.2±43.6	111.5±27.9	115.9±17.3	111.7±22.4	104.2±17.3	104.6±13.8	112.5±14.1	-	113.8±16.3	114.6±15.5
	HES	114.2±28.5	113.7±29.6	116.4±21.1	118.5±26.2	113.3±12.0	106.9±12.1	105.3±12.6	109.3±13.1	102.8±7.13	115.5±13.2	124.8±17.7
DAP (mmHg)	RF	64.3±22.1	67.5±17.4	59.9±12.9	59.9±7.7	58.3±9.2	55.3±7.4	54.8±5.8	56.4±8.2	-	57.5±8.6	55.3±9.5
	HES	63.1±17.5	62.0±18.2	61.2±11.1	61.9±12.0	58.9±7.4	56.1±7.4	55.0±5.8	58.96.1	53.5±2.4	59.0±8.0	59.5±9.3
PP (mmHg)	RF	50.9±29.9	51.7±28.9	51.7±20.7	56.0±14.6	54.4±11.4	53.3±19.0	53.7±15.3	56.1±12.3	-	56.3±16.6	59.3±17.5
	HES	51.1±15.6	51.7±15.6	55.2±13.3	56.6±17.2	53.5±16.4	53.6±12.8	52.1±14.0	50.4±9.8	49.3±6.7	56.5±12.6	65.3±11.6#
MAP (mmHg)	RF	82.4±32.2	85.6±28.3	75.1±16.5	80.0±9.5	76.8±12.6	74.2±10.4	73.4±8.5	75.5±8.9	-	76.6±9.0	75.7±9.4*
	HES	81.5±19.1	80.4±20.0	81.1±15.2	82.9±17.4	78.3±7.3	75.1±9.6	72.9±8.5	76.3±10.1	71.3±3.5	78.7±8.8	83.8±11.4
HR (min^{-1})	RF	69±11	71±10	68±8	66±10	69±12	63±7	66±10	68±9	-	71±10	71±9
	HES	71±13	69±9	74±17	73±12	71±9	72±12	73±12	76±11	82±18	73±14	78±13#
CI ($l \cdot min^{-1} \cdot m^{-2}$)	RF	2.66±0.56	2.76±0.71	2.70±0.56*	2.67±0.52	2.83±0.32	2.56±0.27	2.63±0.42	2.91±0.75	-	2.83±0.40	3.29±0.90#
	HES	2.77±0.54	2.75±0.54	3.22±0.99#	3.01±0.56	3.01±0.57	3.07±0.82	3.13±0.77	2.91±0.32	3.39±0.70	3.02±0.63	3.76±0.86#

Data were recorded after instrumentation at baseline (T_0), at incision (T_i), then hourly until the end of the surgery (T_{es}) and 24 hours after T_0 (T_{24}). Data are presented as mean ± standard deviation. * $P < 0.05$ significant difference between groups. # $P < 0.05$ significant difference from T_0. SAP=systolic blood pressure, DAP=diastolic blood pressure, PP=pulse pressure, MAP=mean arterial pressure, HR=heart rate, CI=cardiac index, PPV=pulse pressure variability, SVV=stroke volume variation, SVI= stroke volume index, SVRI= systemic vascular resistance index, dPmax= index of left ventricular contractility.

Table 6. Hemodynamic parameters in the crystalloid (Ringerfundin, RF)- and colloid (hydroxyethyl starch, HES)-treated groups during the study period. *(Continued)*

	Group	T_0	T_i	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_{es}	T_{24}
PPV (%)	RF	13.07±6.98	9.67±5.00	9.47±2.72	9.93±3.59	10.87±2.36*	10.14±2.73	9.93±3.50	9.81±5.96	-	8.40±3.36	11.33±4.72
	HES	12.40±7.50	10.0±4.30	9.20±5.14	9.27±5.50	8.27±4.28	10.86±7.09	8.09±3.02	8.43±2.76	8.25±2.63	9.80±6.78	9.71±3.86
SVV (%)	RF	14.00±7.17	11.67±5.68	11.67±6.04	10.13±4.61	12.40±5.19	10.86±4.43	11.71±5.01	11.00±4.73	-	10.20±5.63	14.83±7.76
	HES	13.20±7.24	10.27±3.13	10.47±5.69	9.80±5.04	11.47±10.63	14.86±7.73	11.18±7.24	11.71±7.67	11.75±7.89	13.33±7.84	8.57±2.07
SVI (ml/m^2)	RF	36.53±8.69	39.60±7.94#	40.93±6.44	40.00±6.88	41.00±5.01#	40.14±4.84	40.00±4.87	41.73±3.90	-	39.73±5.89	40.70±14.37
	HES	40.40±9.38	40.60±8.52	43.20±7.73	40.66±4.53	41.13±4.86	41.71±6.09	41.81±4.93	41.71±5.41	42.50±2.52	42.13±4.10	48.22±9.83#
SVRI ($dyn \times s\ cm^{-5}\ m^{-2}$)	RF	2503±682*	2378±699	2040±578	2236±484	2069±304#	2113±402	2130±390	1926±370	-	2028±420	2142±702
	HES	1870±627	1880±669	1922±488	2046±459	1967±382	1896±484	1900±519	1871±656	1614±578	2007±450	1745±473
dPmax ($mmHg\ s^{-1}$)	RF	697±295	671±345	611±354	708±420#	674±290	645±347	696±263	618±279	-	801±280	761±180
	HES	563±207	569±215	583±150	622±201	576±160	583±184	621±307	712±238	590±141	687±237	870±189#
T (ºC)	RF	36.1±0.9	36.2±0.9	35.9±0.8	35.7±0.8#	35.8±0.9	35.9±0.8	36.1±0.9	36.4±0.5	-	36.3±1.0	36.6±0.6
	HES	36.3±0.9	36.1±0.8	36.0±0.8#	35.8±0.7#	35.9±0.8#	35.9±0.9	36.2±0.8	36.2±0.7	36.1±0.7	36.1±0.9	36.9±0.5

Data were recorded after instrumentation at baseline (T_0), at incision (T_i), then hourly until the end of the surgery (T_{es}) and 24 hours after T_0 (T_{24}). Data are presented as mean ± standard deviation. * $P < 0.05$ significant difference between groups. # $P < 0.05$ significant difference from T_0. SAP=systolic blood pressure, DAP=diastolic blood pressure, PP=pulse pressure, MAP=mean arterial pressure, HR=heart rate, CI=cardiac index, PPV=pulse pressure variability, SVV=stroke volume variation, SVI= stroke volume index, SVRI= systemic vascular resistance index, dPmax= index of left ventricular contractility.

Table 7. Respiratory variables in the crystalloid (Ringerfundin, RF)- and colloid (hydroxyethyl starch, HES)-treated groups during the study period

	Group	T_0	T_i	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_{es}	T_{24}
Sat. (%)	RF	98.5±0.9	98.5±1.1	98.3±1.2	98.1±1.0	98.4±1.2	98.2±1.1	98.4±1.7	99.4±0.8	-	99.2±1.1	99.1±1.2
	HES	97.9±1.9	97.6±2.1	97.9±1.6	97.7±1.7	97.7±1.3	98.2±1.3	97.9±1.6	97.7±1.6	98.0±1.6	98.7±1.0	99.1±1.0#
FiO_2 (%)	RF	42.9±5.6	42.3±4.6	41.5±5.2	39.7±3.2	39.3±4.1	40.2±2.8	40.7±4.5	45.4±18.7	-	48.6±20.0	32.8±10.7#
	HES	51.7±16.1	49.5±17.0#	46.79.4#	43.4±8.1#	44.6±11.5#	47.9±19.2	50.9±20.8	54.9±27.0	61.3±34.7	63.2±29.1	36.1±10.5#
PEEP (cmH_2O)	RF	3.6±1.5	4.0±1.1	4.0±1.4	4.2±1.3	4.2±1.5	4.4±1.4	4.5±1.4	4.5±1.8	-	4.2±1.3	-
	HES	3.1±1.8	3.0±1.8	3.9±1.1	3.9±1.2	3.9±1.2	3.9±1.0	3.9±0.9	4.3±1.5	3.3±0.5	4.2±1.7	3.2±2.0
TV (ml)	RF	504.3±96.3	496.3±150.8	499.6±79.5	497.0±113.2	518.7±82.5	515.4±88.8	507.4±111.1	499.3±141.4	-	460.3±158.7	-
	HES	464.9±113.4	457.6±78.7	503.1±101.8	514.2±82.0	509.7±101.5	528.5±104.8	476.4±55.0	468.1±43.1	519.3±129.5	519.5±125.1	651.3±68.5
RR (min^{-1})	RF	12±2	12±2	13±2	13±2	13±2	13±2	14±2	14±2	-	14±2	17±4
	HES	12±3	12±2	13±2	13±2	14±2	13±3	13±2	14±2	12±4	12±4	15±3
$EtCO_2$ (mmHg)	RF	31.6±3.7*	33.1±2.9*	32.6±3.4	31.9±3.8	30.7±4.2	30.6±4.8	31.6±5.9	32.3±4.5	-	32.9±4.4	-
	HES	35.3±3.6	36.2±4.1	33.9±4.3	32.9±3.6	32.4±4.4#	31.6±4.1	32.1±3.9	33.4±2.4	35.5±2.6	32.7±4.8	-
MAC	RF	1.0±0.3	1.1±0.3#	1.2±0.3#	1.3±0.5#	1.1±0.2	1.1±0.2	1.1±0.2	1.1±0.3	-	0.9±0.3	-
	HES	1.0±0.2	1.1±0.1#	1.2±0.2#	1.2±0.2	1.8±2.3	1.1±0.3	1.1±0.3	1.1±0.3	1.0±0.2	0.8±0.3	-

Data were recorded after instrumentation at baseline (T_0), at incision (T_i), then hourly until the end of the surgery (T_{es}) and 24 hours after T_0 (T_{24}). Data are presented as mean ± standard deviation. * $P < 0.05$ significant difference between groups. # $P < 0.05$ significant difference from T_0. Sat.= saturation, FiO_2= fraction of inspired oxygen, PEEP= positive end-expiratory pressure, TV= tidal volume, RR= respiratory rate, $EtCO_2$= end-tidal carbon dioxide, MAC= minimum alveolar concentration.

Respiratory data are summarised in Table 7. Overall, all parameters showed similar values in both groups. Although end-tidal CO_2 was significantly elevated in the HES group at T_0 and T_i, it still stayed within the normal range.

4.2.3. Blood gas parameters

All parameters remained within the physiological normal range throughout the observation period and, although there were certain statistically significant differences observed, these can be regarded as clinically nonrelevant (Table 8). Haemoglobin concentration in the HES group showed a significant decrease over time, without the need for blood transfusion.

Oxygen consumption and oxygen delivery were more or less stable throughout the study and followed similar patterns in both groups. Oxygen extraction changed accordingly with no major difference between the groups.

Table 8. Blood gas parameters in the crystalloid (Ringerfundin, RF)- and colloid (hydroxyethyl starch, HES)-treated groups during the study period.

	Group	T_0	T_i	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_{es}	T_{24}
a pH	RF	7.43±0.20	7.42±0.19	7.35±0.05	7.36±0.06	7.37±0.03	7.36±0.04	7.36±0.04	7.34±0.09	-	7.33±0.08	7.38±0.08
	HES	7.33±0.00	7.33±0.03	7.34±0.03	7.36±0.02	7.35±0.02	7.36±0.03	7.34±0.03	7.35±0.02	7.33±0.03	7.34±0.03	7.43±0.07#
a PCO_2 (mmHg)	RF	44.2±8.3*	44.5±8.1*	46.8±6.8	45.4±7.3	42.3±4.9	43.2±7.5	43.2±7.5	46.6±14.9	-	47.9±12.9	43.2±3.0
	HES	50.4±4.6	50.4±4.6	47.3±4.4	44.8±3.9#	44.9±3.2#	44.4±3.9	46.4±5.1	45.7±2.2	51.4±4.2	47.1±6.3	41.0±8.9#
a PO_2 (mmHg)	RF	148.0±46.2	155.3±41.0	137.3±41.2	138.8±30.9	143.7±28.9	152.0±34.6	142.7±28.7	142.7±17.9	-	145.5±33.5	151.1±49.3
	HES	174.1±54.9	174.1±54.9	153.1±71.2	131.6±39.2#	130.2±33.4#	145.9±56.4	148.0±61.7	123.1±42.7	151.3±100.1	184.4±71.2	138.6±41.0
a BE (mmol l^{-1})	RF	-0.7±1.6	-0.9±1.6	-0.7±1.3	-1.1±1.5	-1.5±1.5 #	-1.6±1.4	-1.9±1.2	-2.3±1.5	-	-2.0±1.3	1.2±1.6#
	HES	-1.1±1.4	-1.1±1.4	-1.0±1.4	-1.2±1.1	-1.2±1.4	-1.2±1.3	-1.3±1.1	-0.8±0.9	0.2±1.1	-1.2±1.4	1.0±1.5#
a HCO^-_3 (mmol l^{-1})	RF	24.8±1.7	24.6±2.0	25.1±1.5	24.4±1.7	23.7±1.7#	23.8±2.2	23.6±2.0	23.6±2.0	-	24.1±1.8	26.0±1.5
	HES	25.5±1.4	25.5±1.5	25.0±1.5	24.6±1.4#	24.4±1.4#	24.3±1.4	24.5±1.4	24.9±0.8	26.5±1.0	24.7±1.9	25.3±2.5
a SO_2 (%)	RF	98.5±1.1	98.6±1.1	98.3±1.0	98.6±0.7	98.7±0.6	98.8±0.7	98.7±0.7	99.4±1.6	-	98.6±0.8	98.7±0.8
	HES	98.8±1.0	98.8±1.0	98.4±1.1	98.3±1.0	98.4±0.7	98.5±0.8	98.4±0.9	97.7±1.6	98.1±1.3	99.0±0.7	98.6±0.9
a lactate (mmol l^{-1})	RF	1.1±0.4	1.2±0.5	0.9±0.2	1.0±0.3	1.1±0.4	0.9±0.2	1.1±0.3	0.9±0.4	-	1.0±0.3	1.0±0.4
	HES	1.3±1.0	1.3 1.0	1.0 0.3	1.0±0.4	1.1±0.5	1.1±0.6	1.3±0.9	1.6±1.4	0.8±0.4	1.6±1.1	1.3±0.6
a Hb (g dl^{-1})	RF	11.7±1.1	11.7±1.1	11.9±1.3	11.8±1.3	11.9±1.5	11.1±1.4	11.3±1.3	11.2±1.7	-	11.0±1.4	10.6±1.1#
	HES	12.6±1.3	12.6±1.3	11.3±1.3#	10.9±1.5#	10.4±1.5#	10.2±2.0	10.1±2.2	10.1±1.3	9.8±1.2	10.1±2.2	10.4±1.2#
cv PCO_2 (mmHg)	RF	51.45±8.2	50.8±8.8	51.7±6.5	50.2±5.9	48.5±5.4	48.6±4.6	49.0±5.7	53.1±14.9	-	52.9±13.2	50.5±3.3
	HES	55.6±5.0	55.6±5.0	52.6±4.3	50.2±4.0#	50.0±3.2#	49.2±4.1	51.8±5.2	51.6±2.9	55.4±3.2	51.9±5.8	46.8±8.6#

Table 8. Blood gas parameters in the crystalloid (Ringerfundin, RF)- and colloid (hydroxyethyl starch, HES)-treated groups during the study period. *(Continued)*

	Group	T_0	T_i	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_{es}	T_{24}
$ScvO_2$ (%)	RF	81.8±6.4	83.9±6.5	83.9±6.0	83.5±3.2	81.6±4.0	79.6±4.5	79.3±5.4	80.8±5.2	-	81.3±5.2	78.9±3.1
	HES	84.9±5.0	84.9±5.1	85.7±5.6	84.1±4.8	83.5±3.6	83.3±5.0	82.2±3.6	84.1±3.3	83.6±3.1	82.2±4.3	79.6±4.6#
CO_2 gap (mmHg)	RF	6.7±3.4	5.3±2.9	5.3±2.3	6.0±1.1	6.0±2.1	6.4±2.1	6.4±2.1	6.5±2.2	-	6.1±2.0	7.3±2.7
	HES	5.2±2.1	5.2±2.1	5.4±2.2	5.4±1.7	5.1±1.5	4.8±1.4	5.4±2.6	6.2±2.1	4.0±3.0	4.9±2.9	5.8±2.0
PaO_2/FiO_2	RF	366.0±121.1	378.1±97.2	345.6±115.0	351.8±97.2	362.0±97.0	383.7±104.2	357.9±100.4	345.7±102.5	-	320.3±116.0	511.1±242.1
	HES	348.2±125.9	375.7±160.9	349.8±195.1	314.7±102.7	308.7±99.4	331.8±146.3	317.0±125.0	297.9±132.0	265.0±92.5	327.4±170.2	406.1±168.0#
DO_2I ($ml \cdot min^{-1} \cdot m^{-2}$)	RF	445.5±106.5	476.1±124.6	438.4±82.3	423.3±91.6	451.0±90.4	394.8±80.6	410.5±93.8	447.7±87.0	-	405.4±100.8	476.2±132.1
	HES	495.1±118.0	492.2±106.5	506.7±167.9	449.8±91.3	431.1±92.2	424.1±102.2	427.2±109.2	439.0±102.7	455.3±107.7	428.2±83.5	543.0±172.3
VO_2I ($ml \cdot min^{-1} \cdot m^{-2}$)	RF	76.8±32.5	73.4±35.5	64.5±26.8	89.0±71.0	78.6±21.5	78.7±24.8	80.0±23.6	82.5±30.2	-	77.4±29.0	94.6±26.0
	HES	70.5±30.4	69.3±27.3	61.2±22.3	65.0±25.6	66.5±23.3	66.7±28.3	69.2±16.0	64.3±22.2	68.5±24.5	71.6±29.2	102.9±34.8#
ERO_2 (%)	RF	17.1±6.4	15.1±6.2	14.8±5.7	21.5±18.3	17.5±3.9	19.7±4.4	19.7±5.5	18.3±5.2	-	17.8±5.0	20.2±3.1
	HES	14.2±4.8	14.2±4.8	13.1±5.4	14.6±4.5	15.3±3.4	15.6±5.2	16.6±3.8	14.5±3.2	15.0±3.2	17.2±4.2	19.5±5.0#

Data were recorded after instrumentation at baseline (T_0), at incision (T_i), then hourly until the end of the surgery (T_{es}) and 24 hours after T_0 (T_{24}). Data are presented as mean ± standard deviation. * $P < 0.05$ significant difference between groups. # $P < 0.05$ significant difference from T_0. Parameters measured in the arterial blood samples: a pH, a PCO_2= arterial carbon dioxide partial pressure, a PO_2= arterial oxygen partial pressure, a BE= arterial base excess, a HCO^-_3= arterial bicarbonate, a SO_2= arterial oxygen saturation, a lactate= arterial lactate, a Hb= arterial hemoglobin; parameters measured in central venous blood samples: cv PCO_2= central venous carbon dioxide partial pressure, cv PO_2= central venous oxygen partial pressure, $ScvO_2$= central venous oxygen saturation, CO_2 gap= central venous-to-arterial pCO_2-gap, PaO_2/FiO_2 = the ratio of arterial oxygen partial pressure to fractional inspired oxygen, DO_2I= oxygen delivery index, VO_2I= oxygen consumption index, ERO_2= oxygen extraction.

4.2.3. Total amount of the intra-operative and ICU medications

As listed in Table 9, the Ringerfundin group required significantly more boluses and greater total amounts of fluid. Blood loss did not differ between the groups. During surgery, the Ringerfundin group was given 1.5 times more boluses of fluid than the HES group. There were no significant differences in the postoperative period. Nearly half of the patients required vasopressors (Ringerfundin, *n*= 8; HES, *n*= 7) and inotropic support (Ringerfundin, *n* = 6; HES, n = 7), without significant difference in the required doses between the groups. Total amount of anaesthetic and analgesic agents was similar in both groups.

Table 9. Total amount of intraoperative (IOP) medications in the crystalloid (Ringerfundin, RF)- and colloid (hydroxyethyl starch, HES)-treated groups

Fluid balance	RF (*n*=15)	HES (*n*=15)	(*P*=)
IOP Total Maintenance fluid (RF ml)	731.2 ± 621.5	653.2 ± 229.3	0.654
IOP Boluses (ml)	1850.0±900.4	1150.0 ± 580.9	0.017*
IOP Boluses (events)	7.4±3.6	4.6±2.3	0.017*
IOP Total fluid (ml)	2581.2±986.2	1803.2±497.9	0.011*
IOP Blood loss (ml)	150.0[100.0-225.0]	250.0[100.0-400.0]	0.346
Vasopressors, Inotropes			
IOP Noradrenalin (mcg)	10.0[0.0-781.4]	0.0[0.0-450.0]	0.870
IOP Dobutamine (mg)	0.0[0.0-35.5]	0.0[0.0-79.5]	0.967
Anesthetics, Analgesics			
IOP Propofol (mg)	172.0±62.7	190.7±62.3	0.420
IOP Morphine (mg)	15.0[13.0-20.0]	20.0[12.0-20.0]	0.512
IOP Fentanyl (μg)	100.0[0.0-100.0]	100.0[100.0-200.0]	0.202
IOP Rocuronium (mg)	132.0±50.6	139,0±41.2	0.681

Table 9. Data are presented as mean ± standard deviation or median [IQR]. * $P < 0.05$ significant difference between groups.

4.2.4. Microcirculation and corresponding macrohaemodynamics

As evidenced by laser-Doppler flowmetry, baseline perfusion values were similar at the flap areas (*in situ*, before harvesting) and at the control sites in both groups (at 35℃ and 44℃) (Figs. 9 and 10). During reperfusion, however, significantly higher tissue perfusion values were observed at the free flap sites in both groups than those observed at baseline or at the control areas (at corresponding timepoints) at 35℃ (Fig. 9). A significant difference in the perfusion of the free flaps areas was observed only in the ninth hour of reperfusion between the groups when perfusion values appeared to be higher in the Ringerfundin group. Heat provocation (to 44℃) induced increases in tissue perfusion only at the control areas, whereas this effect was missing in the flaps during reperfusion in both groups (Fig. 10). In the macrohaemodynamic values, a significant difference was found in the DAP and PPV during the second hour of reperfusion (R_2), but these changes were not accompanied by significant changes in microcirculatory perfusion at any sites. Changes in macrohaemodynamic parameters at different reperfusion measurement points varied according to the surgical section (Table 10).

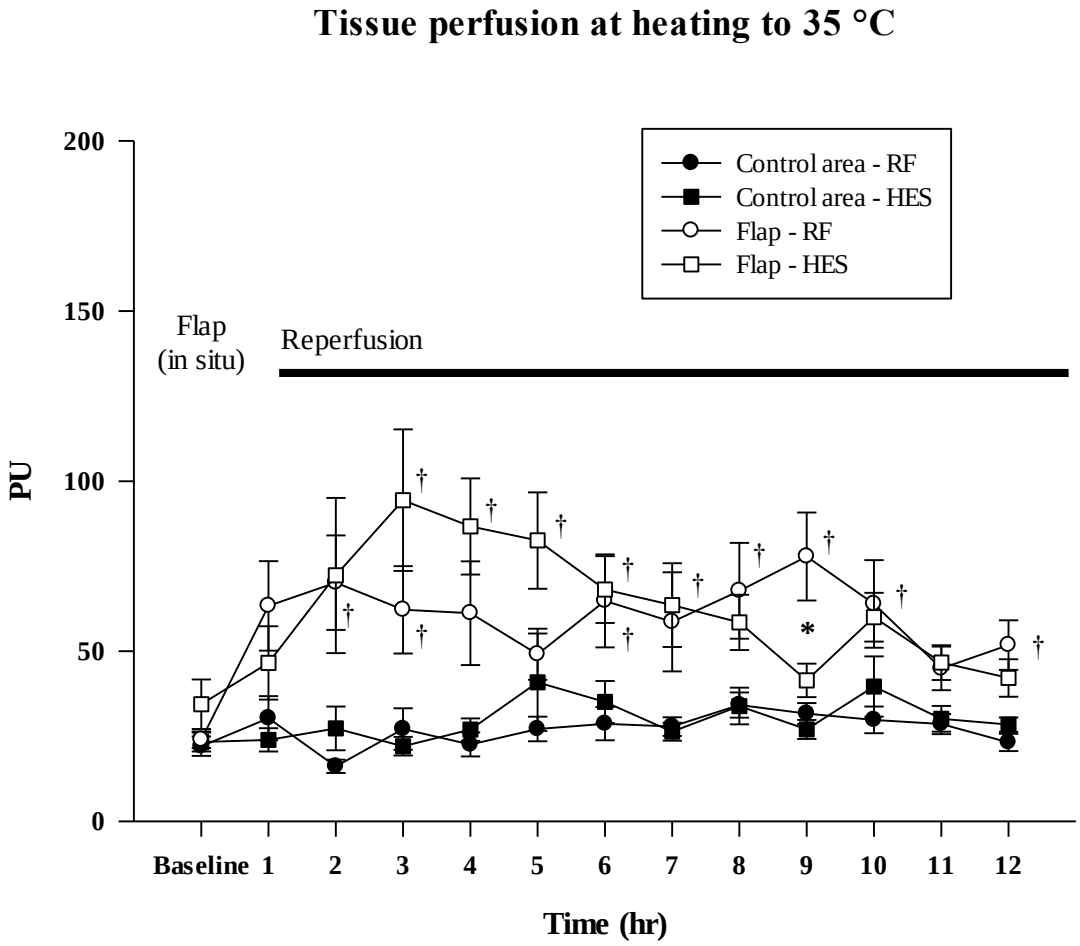

Figure 9.:Perfusion is expressed as the ratio of the perfused units at 44℃/PU without heating (i.e. at original temperature). The figure shows tissue perfusion on heating to 35℃ in the forearm flap and at the control area (skin at the deltoid region in the crystalloid (RF) and colloid (HES) treated patients at different time-points of the study. Recordings were taken when the flap was prepared (Rbsl), 1 h after reperfusion and continued hourly for up to 12 h. Data are presented as mean ± SD. ¬* $P < 0.05$ significant difference between groups. † $P < 0.05$ vs. Rbsl.

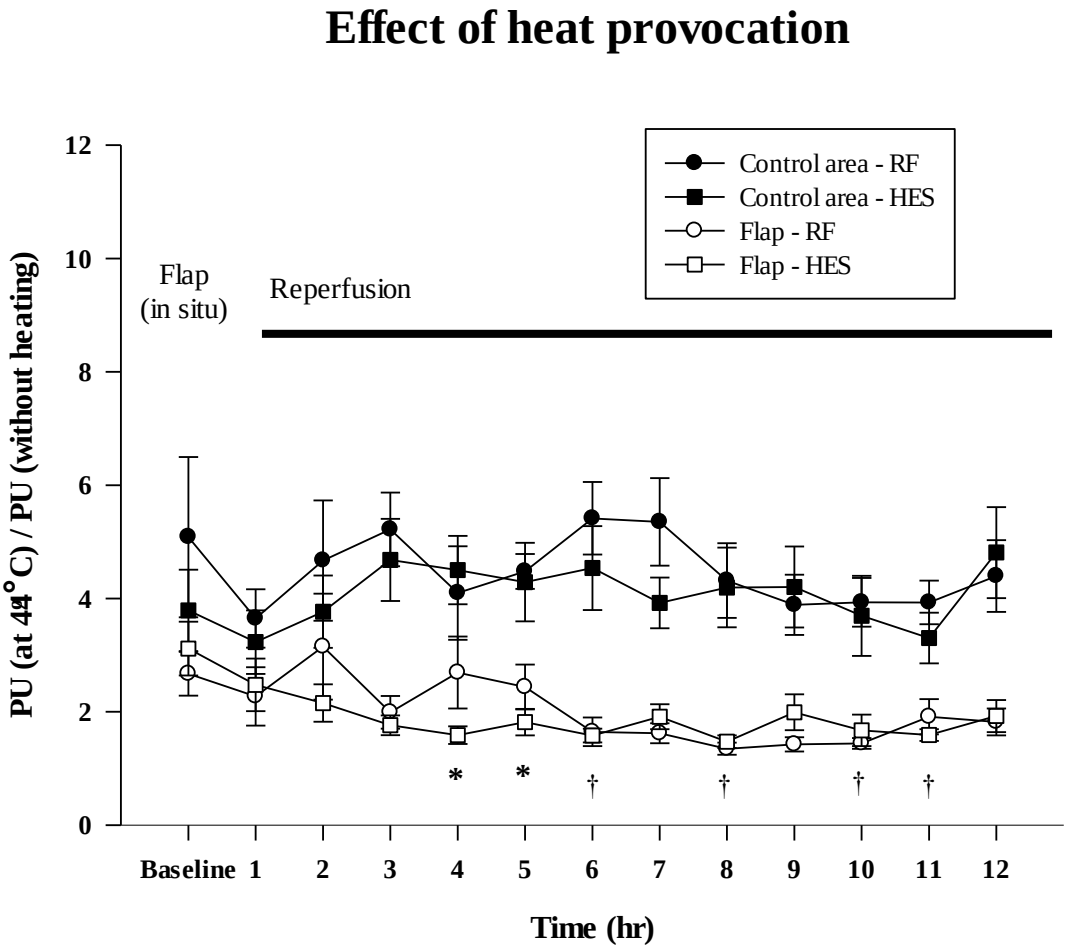

Figure 10.:Perfusion is expressed as the ratio of the perfused units at 44℃/PU without heating (i.e. at original temperature). The figure shows the effects on perfusion at the control site (skin at the deltoid region) and in the forearm flap in the crystalloid (RF) and colloid (HES) treated patients at different time-points. Recordings were taken when the flap was prepared (R_{bsl}), 1 h after reperfusion and continued hourly for up to 12 h. Data are presented as mean ± SD. * $P < 0.05$ significant difference between groups. † $P < 0.05$ vs. $R_{bsl.}$

Table 10. Hemodynamic parameters in the crystalloid (Ringerfundin, RF)- and colloid (hydroxyethyl starch, HES)-treated groups at baseline and during reperfusion (at matching time-points with the microcirculatory measurements)

	Group	R_{bsl}	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}	R_{11}	R_{12}
SAP (mmHg)	RF	122±28	126±21	133±31	131±28	126±22	125±17	122±22	119±20	119±28	121±18	120±15	119±20	119±19
	HES	118±21	117±18	120±30	147±25†	140±31	129±27	123±19	114±15	115±14	116±17	111±21	123±22	119±16
DAP (mmHg)	RF	60±11	63±11	66±12*	64±15	63±19	66±19	65±15	62±15	57±12	58±12	60±16	56±12	56±11
	HES	61±9	58±8	57±7	68±9	62±9	61±8	58±9	56±7	56±9	55±7	52±8†	54±8	53±8†
MAP (mmHg)	RF	80±13	86±19	88±19	87±18	84±18	88±15	86±12	83±12	81±18	80±14	80±13	80±16	80±14
	HES	83±13	79±12	79±15	101±26†	93±30	85±15	81±11	76±9	77±10	76±10	72±10†	78±11	78±10
HR (min^{-1})	RF	68±12	66±10	72±12	71±11	72±13	70±10	69±9	69±12	69±12	69±11	68±12	68±12	68±12
	HES	74±9	71±10	76±14	84±25	78±21	82±25	78±24	79±22	76±19	77±17	74±17	75±17	75±16
CI ($l\ min^{-1}\cdot m^{-2}$)	RF	2.8±0.5	3.0±0.3	3.0±0.5	3.2±0.9	3.2±0.7	3.2±0.8	3.1±0.8	3.0±0.7	3.2±0.8	3.2±0.8†	3.0±0.6	3.2±0.7	3.2±0.8†
	HES	3.1±0.5	3.1±0.6	3.2±0.6	3.6±1.2†	3.6±1.2†	3.6±1.3	3.3±0.8	3.1±0.6	3.0±0.6	3.2±0.7	3.2±0.6	3.3±0.8	3.4±0.7
PPV (%)	RF	9.5±2.5	8.4±3.6	7.5±2.9*†	8.0±3.6	8.6±6.8	9.8±6.9	9.5±6.2	8.4±5.3	9.1±5.8	9.3±4.0	8.0±3.0	9.1±3.7	9.0±2.7
	HES	8.6±2.7	9.5±4.2	10.3±3.5	12.0±6.7	11.4±5.5	12.2±6.7	11.2±6.4	11.7±5.9	11.0±5.1	10.5±3.8	11.0±4.4	11.0±5.0	10.1±3.5
SVI ($ml\ m^{-2}$)	RF	37.8±10.9	41.7±6.4	42.4±7.1	45.0±7.7†	42.6±8.1	43.5±10.1	44.7±8.8†	43.2±9.2	45.2±9.6†	46.7±9.0†	45.3±10.5†	46.8±8.3†	48.2±9.1†
	HES	42.5±6.2	43.5±6.0	43.5±5.9	45.7±6.3	46.8±6.4	44.0±5.6	42.3±4.4	40.8±4.9	41.5±5.3	42.3±5.5	43.0±6.4	45.7±6.4	48.2±7.9

Table 10. Data were recorded after the flap was prepared (R_{bsl}), then 1 hour after reperfusion and continued hourly for up to 12 hours (R_1- R_{12}). Data are presented as mean ± standard deviation. * $P < 0.05$ significant difference between groups. †$P< 0.05$ significant difference from R_{bsl}. SAP=systolic blood pressure, DAP=diastolic blood pressure, PP=pulse pressure, MAP=mean arterial pressure, HR=heart rate, CI=cardiac index, PPV=pulse pressure variability, SVV=stroke volume variation, SVI= stroke volume index, SVRI= systemic vascular resistance index, dPmax= index of left ventricular contractility.

5. DISCUSSION

The main findings of our animal experiment study are that stable hemodynamic parameters were achieved by significantly more RF than HES boluses and that the VR ratio was more than three times higher in the RF group compared to the HES group. In our randomised human study we found that patients in the Cryst group required more fluid than patients in the Coll group, without any significant difference in the macrocirculation and microcirculation. To detect hypovolemia, advanced haemodynamic monitoring was applied in order to minimise the possibility of diagnostic errors and administer fluid boluses only when hypovolemia was strongly supported by physiological parameters.

5.1. Haemodynamic assessment

Over the last decades there were several clinical trials published on goal-directed fluid resuscitation.[5] Delay or inadequate management will inevitably lead to hypoperfusion, tissue hypoxia or edema, and fluid overload, leading eventually to multiple organ failure, seriously affecting outcomes.[51] In these studies hemodynamic goals showed a great variability. The most frequently used parameters to guide fluid management were CI, SV, SVV, PPV, CVP, MAP, echo-derived dynamic indices, pulmonary artery occlusion pressure, DO_2, and oxygen extraction ratio.[5] Unfortunately, "fixed" treatment protocols neglect the patient's individual needs, and what is shown to be beneficial for a given population may not be so for the individual patient. However, applying a multimodal, contextualized, and personalized management could potentially overcome this problem. [51]

5.1.1. Measures of Oxygen Debt

According to a large international study (FENICE study), physicians frequently use inadequate indices to guide fluid management in intensive care units. Goal-directed and "restrictive" infusion therapies have been recommended by guidelines over "liberal" approaches for several years.[2,51] The main aim of hemodynamic management is to restore the balance between oxygen demand and supply at the tissue level. Central venous oxygen saturation, venous-to-arterial carbon dioxide gap (dCO_2) and lactate have all been proposed as potential resuscitation targets in hemodynamically unstable patients.

$ScvO_2$ is a frequently used parameter and is routinely used in patients with any shock. Changes in $ScvO_2$ can potentially indicate clinically significant anemia, hypovolemia, and impaired myocardial function and can be affected by medications, body temperature, or a

combination of any other factors that are able to influence the VO_2/DO_2 relationship.[52] It is known that both low and high $ScvO_2$ values are associated with higher mortality in patients with sepsis.[53,54] Although the three previous large, prospective, and multicenter randomised studies (PROCESS, ARISE, PROMISE) [55–57] failed to demonstrate any mortality benefits of an $ScvO_2$-based approach. This may have been due in part to the protocols, which applied fixed values of certain parameters as targets for the whole study population and neglected the patients individual needs. Furthermore, a contextualised approach was also missing from the protocols, hence the targeted parameters were not put in the context of the overall hemodynamic picture (including measures of VO_2/DO_2) that is a prerequisite for a personalised approach.

Another blood flow-related variable is central venous-to-arterial carbon dioxide partial pressure difference. From a physiological standpoint, adapting the Fick principle to carbon dioxide production and elimination, the following equation describes the dCO_2 gap.[58]

$P(v\text{-}a)CO_2 = VCO_2 / CO$

where VCO_2 is CO_2 production, and CO is cardiac output. This shows the indirect relationship between dCO_2 gap and CO and explains why an increased dCO_2 gap usually corresponds to low flow states. A recent meta-analysis suggested that increased dCO_2 (>6 mmHg) was associated with increased mortality, elevated lactate levels, and a lower CI in ICU patients.[59]

Lactate is an important marker of tissue metabolism, which is a parameter of tissue hypoperfusion/hypoxia. Furthermore, it is a recommended resuscitation target in septic shock [60], and its peak concentration and persistent hyperlactatemia after resuscitation is regarded as a remarkable prognostic factor of unfavorable outcomes in shock.[61,62] It is important to mention that according to the latest evidence, sepsis-related lactate production is not solely due to tissue hypoxia or hypoperfusion; therefore, the degree of change in lactate levels or high lactate in sepsis are not always a true reflection of impaired oxygen delivery and tissue hypoperfusion.[54,63]

Optimisation of global cardiovascular dynamics to maintain or restore tissue perfusion and oxygenation is a promising concept to improve postoperative outcome in patients having surgery.[64] Intraoperative hypotension is common in patients having noncardiac surgery under general anaesthesia, and large cohort studies have shown an association between the

severity and duration of intraoperative hypotension and myocardial injury, acute kidney injury, and death.[65,66] Therefore, 'sensitive, specific, and continuous measures of cellular function to evaluate arterial pressure management in a physiologically coherent manner' need to be developed.[67]

5.1.2. The Individualized Concept

Although this wasn't the aim of the current studies but briefly summarizing the concept of individualized hemodynamic management maybe useful. Multimodal, individualised, contextualised management of intraoperative cardiovascular dynamics. Whenever an alarming signal occurs, all potential causes should be considered to determine whether the alarming findings represent clinically relevant pathophysiology.[68]

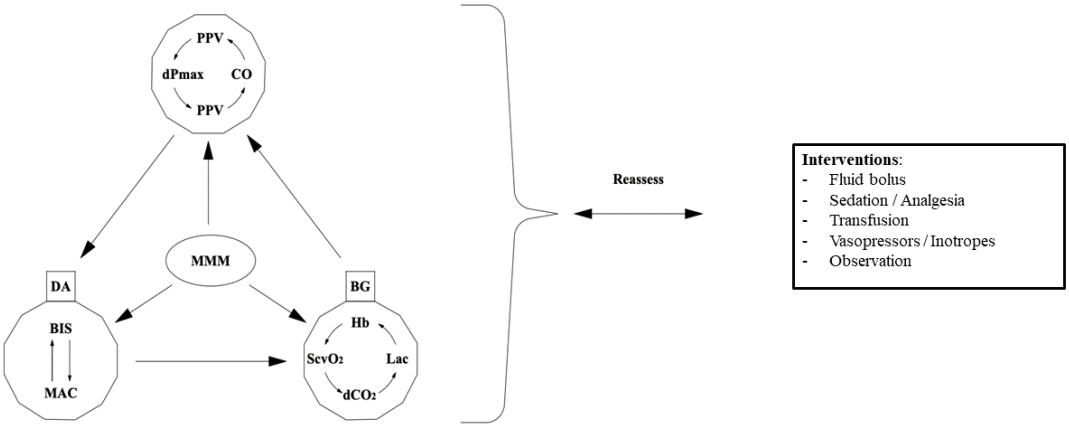

Figure 11.:MMM: Multimodal monitoring. If considered clinically relevant, interventions to treat should be performed followed by frequent reassessment (reassess). If the alarming signal is regarded as false alarm, then observe and reassess later. HM: hemodynamic monitoring, PPV: pulse pressure variation CO: cardiac output; MAP: mean arterial pressure, dPmax: left ventricular contractility; DA: depth of anaesthesia (BIS: bispectral index, MAC: minimum alveolar concentration, etc.); BG: Blood gas parameters, dCO_2: central venous-to arterial CO_2-gap, Hb: haemoglobin, Lac: lactate; $ScvO_2$: central venous oxygen saturation.

5.2. Crystalloids vs. colloids – macrocirculation

Several large, multicentre, randomised clinical trials were published mainly in critically ill patients. Most of these compared crystalloids with HES and concluded that there is no difference, or they observed worse outcome in the HES group.[20–22] On the basis of these results, current guidelines recommend strongly against the use of HES especially in septic patients.[69] Regarding the VR effects, in these trials, there was a similar VR ratio for Cryst and Coll, which is summarized in Table 1. Based on these results, a common view was formed that starch solutions do not have a significantly higher potency for volume expansion as compared to Cryst, but carry a greater risk of renal dysfunction and mortality. However, it is important to note that none of these trials used detailed hemodynamic monitoring. The administration of i.v. fluids was mainly based on the clinicians' subjective decision, or on a single parameter such as heart rate, blood pressure, central venous oxygen saturation, urine output, and lactate level, or some kind of a combination of these, none of which are a good predictors of fluid responsiveness. Linton et al. nicely showed in a postoperative critical care population that the relationship between MAP and oxygen delivery is very poor.[70]

5.2.1 Volume-replacement ratio

Our first aim was to perform a bleeding-resuscitation animal experiment with detailed hemodynamic monitoring, predefined end points, and a pragmatic protocol. During the experiment, hemodynamic changes did not show clinically relevant differences between the two groups. Kinetics of CI, SVI, MAP, HR, and GEVI showed similar patterns in both groups with significantly higher values in the HES group at the end of the experiment. The higher macro-hemodynamic values in the HES group may be due to the more rapid hemodynamic effects of Coll in general as compared to Cryst. SVV and PPV almost doubled after bleeding in both groups and then returned to baseline values, with both being significantly lower in the HES group. Contractility, as indicated by dPmax values, also showed similar changes in both groups. In other words, we observed a similar hemodynamic course for these animals during the experiment, but the volume required was more than three times higher in the RF group. We detected elevated lactate and extravascular lung water (EVLW) levels from the start. This could have occurred during the preparation process, which may have caused some kind of distress. Nevertheless, EVLW did not reach extremely high values and regarding lactate, pigs can have higher blood lactate levels than humans, ranging from 0.5 to 5.5 mmol l^{-1}.[71,72]

5.2.2 Effects of goal-directed crystalloid vs. colloid fluid therapy on microcirculation during free flap surgery

Although the investigation of renal function was not the goal of our randomised clinical trial, the finding that there was no difference in urine output, and serum creatinine levels remained in the normal range in both groups, with no patient requiring renal replacement therapy in the HES group provides support that there is no current evidence that HES causes renal insufficiency in the peri-operative period.[73,74] The main cause is that the acute elevation in CI induced by a bolus infusion of Cryst is unlikely to last long. When Cryst are administered rapidly, within, a distribution phase will be dominant. Almost half of the induced plasma volume expansion is lost within 10 min. During general anaesthesia except when there is a sudden drop in arterial pressure, which transiently arrests the distribution.[75] The distribution effect becomes lower with the infusion time and is of insignificant consequence for long therapies. Then, the rate of elimination is the main factor determining the plasma volume expansion, which fact has cast doubts over the relative potency of these fluids in several studies.[76–78] Theoretically, Coll have better volume expansion effects, therefore they restore the circulating blood volume and hence DO_2 faster than Cryst do. A fluid challenge study shows that fluid responsiveness is time-dependent and that the issue of optimal timing needs to be addressed.[77,79] The aimed to evaluate whether echocardiographic assessment of the response to fluid challenge could affect the results by Cryst infusions. The question is whether smaller volumes of Coll have any plausible clinical benefit. However it was not the aim of our clinical trial, our data support the theory of Starling's three-compartment model and provided additional information that using Coll may have the benefit of reaching haemodynamic stability two to three times faster compared with Cryst. This difference could potentially be important during fluid resuscitation.[80,81]

5.3. Crystalloids vs. colloids – microcirculation

One of the main causes why the vasculature may behave differently than that described by Starling is the recently discovered role of the GX in the function of the endothelium. A web of membrane-bound glycoproteins and proteoglycans in the luminal side of endothelium has been identified as the GX layer. This compartment consists of many highly sulfated Glycosaminoglycan (GAG) chains providing negative charge for the endothelium. Due to these electrostatic properties, the subglycocalyx space produces a Coll oncotic pressure that

might be an important determinant of vascular permeability and thus fluid balance.[10] The structure and function of the endothelial GX varies substantially among different organ systems and is affected by inflammatory conditions, such as sepsis.[82] Theoretically, with an intact GX, the VR ratio is markedly different for Cryst compared to Coll and may behave as suggested by Starling.[83] This is also supported by other studies, including our current experiment. In a recent trial on healthy volunteers, it was demonstrated that after 1000 ml of Cryst (isotonic saline) or Coll (gelatine and hydroxyaethyl starch) infusion, the latter caused a four times greater increase in blood volume compared to saline, and extravasation was significantly higher after saline infusion: saline 68%, gelatine 21%, and starch 16%.[84]

Among the proteoglycans, syndecans are the main structural elements of GX and thus play a key role in the functional changes of the endothelium.[85] Four subtypes are known, each of which is connected to the cell membrane via a transmembrane domain, but also has cytoplasmic domains.[83,86] Recent clinical studies and animal experiments have confirmed that the increase in plasma levels of syndecans, which also serve as indicators of GX degradation, show a positive correlation with mortality in critically ill patients, and in particular the degradation of syndecan-1 and 3 subtypes increases in various shock states.[87–89] An increase in the plasma level of syndecan-1 in various models with systemic and local damage has been demonstrated in several experiments, mainly on mice.[90] Thus, for example, sepsis [91], ischemia-reperfusion [92], burns [93], pancreatitis [94], surgical intervention [95], hemorrhagic shock [89], and kidney transplantation have both detected an increase in systemic syndecan-1 levels.[96] In hemorrhagic experimental mouse shock model they found that plasma levels of syndecan-1 increased threefold in the Cryst-resuscitated group, two-fold in the albumin-treated group, and showed no significant change in the synthetic Coll-treated group compared to the control group.[89] At the same time, the thickness of the GX was reduced by half in the group treated with Cryst, and by 25% in the group treated with albumin and synthetic Coll compared to the control group.[89]

Correction of systemic haemodynamics should be effective in ameliorating regional and microcirculatory perfusion.[97] The assumption that Coll, compared with Cryst, have superior effects on the microcirculatory blood flow underestimates the importance of haemodynamic stabilisation, and gives all the credit to the better pharmacological properties of Coll.[33,98] Furthermore, most of the studies in the field did not measure cardiac output and its derivatives. Therefore, one cannot exclude that the beneficial effects of Coll on the microcirculation were due to their better volume expansion, which caused an

increased cardiac output, hence better regional blood flow, rather than the features of the Coll molecules per se.

5.3.1. Volume-replacement ratio

In our experiment in healthy pigs, we also found similar differences between the volume expanding effects of RF and HES solutions. This suggests that during the early phase of bleeding, when theoretically the endothelium and the GX are intact, Coll have volume sparing effects compared to Cryst.[72]

5.3.2. Effects of goal-directed crystalloid vs. colloid fluid therapy on microcirculation during free flap surgery

In our trial, microcirculation (as examined by laser-Doppler flowmetry) showed no clinically important difference between the Cryst and Coll groups either when the probe was placed on the control area or on the flap. Because the free flaps lose their innervation (including sympathetic innervation) during harvesting, significantly higher perfusion values can be observed at these sites during reperfusion.[99] Heat induced vasodilation, which requires both intact innervation (mostly mediated by c-afferents) and endothelial function[100], is apparently lost in the free flaps during reperfusion in both treatment groups. This latter reaction, however, is present in the control areas, and reaches similar values in both groups. These results suggest that haemodynamic stability probably has a higher impact on regional microcirculation than the type of solution used to achieve this state. [33,34] Furthermore, intravenously administered vasopressors may affect denervated vessels via the endothelial nitric oxide system [101,102], but our results suggest that norepinephrine can be used safely and does not harm the flap when its administration is controlled by appropriate haemodynamic assessment. Although all flaps survived the study period, we observed that four flaps failed in the HES group and one in the Ringerfundin group within the first 10 days. However, in three cases, flap failure occurred most probably due to surgical complications, but our sample size is too small to draw any firm conclusions.[103]

6. LIMITATIONS

6.1. Volume-replacement ratio

First, in this model, the course of bleeding took place relatively quickly, which was almost immediately followed by resuscitation. Therefore, the results of the current study can only be partially applied to clinical practice. Another limitation is that microcirculation and extravasation of fluid was not monitored or assessed in any way; hence, the measurement of certain GX degradation molecules can only be considered as indirect indicators of GX integrity. A more detailed evaluation would be necessary to prove our concept that Starling's theory worked in this model. Another limitation of our results is that animals remained alkalotic in the HES group as a result of unintentional hyperventilation at Tbsl, T0, and T1. However, whether it interfered with the results to any extent is difficult to tell. High EVLW and lactate levels, which were elevated and remained so throughout, indicate that animal preparation, which required a considerable length of time, was not as gentle as meant to and may have caused some distress. Finally, the long-term effects of fluid resuscitation were not assessed.

6.2. Effects of goal-directed crystalloid vs. colloid fluid therapy on microcirculation during free flap surgery

This study also has limitations. It was a single-centre trial with a relatively small sample size and long-term effects were not evaluated. Examination of the effects of HES on renal function would have been an important issue, but this was not among the aims of the present study. Haemodynamic monitoring was performed with a noncalibrated device. It has been reported that the accuracy and trending ability of noncalibrated devices are moderate or even poor when compared with gold standard technologies applying thermodilution measurements.[104,105] However, such results were mainly reported in critically ill patients, a very different population from that in the present trial. Although awareness and pain can exert a profound effect on haemodynamic variables, depth of anaesthesia monitoring was not used. However, continuous measurement of end-tidal sevoflurane was performed, and we aimed to keep the minimal alveolar concentration above 1.3 to achieve adequate depth of anaesthesia. As regards the type of fluids chosen, comparing Ringerfundin with a balanced HES-solution (Volulyte rather than Voluven) could have been a better choice. Finally, if GX degradation products could have been measured, it could have provided an important explanation for the observed difference in the treatments.

7. KEY MESSAGE OF THE STUDY

7.1. Volume-replacement ratio

- Our data provides experimental evidence that for the same hemodynamic effect, significantly more Cryst than Coll solution is required in healthy pigs.

- The VR ratio was very similar to that described by Starling. Our data also suggests that the Starling's "three-compartment model" requires an intact endothelial GX.

- Therefore, the clinical importance of our results is that Coll may have a place in the very early resuscitation phase, before the GX suffers impairment, to maintain haemodynamic stability faster that could be achieved with Cryst.

7.2. Effects of goal-directed crystalloid vs. colloid fluid therapy on microcirculation during free flap surgery

- In this randomised clinical trial performed during free flap surgery, we found that compared with Coll, larger volumes of Cryst were needed to achieve similar haemodynamic stability.

- There was no difference between the Cryst and Coll groups as far as haemodynamic parameters were concerned nor was there a difference in flap perfusion either.

- Our results indicate that in patients without relevant blood losses and relatively low infused fluid volumes, when haemodynamic stability is maintained with the aid of detailed haemodynamic assessment, there is no measurable benefit of Coll over Cryst.

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REFERENCES

1. Benes J, Kirov M, Kuzkov V, Lainscak M, Molnar Z, Voga G, Monnet X: Fluid Therapy: Double-Edged Sword during Critical Care? Biomed Res Int 2015; 2015:1–14

2. Cecconi M, Hofer C, Teboul JL, Pettila V, Wilkman E, Molnar Z, Rocca G Della, Aldecoa C, Artigas A, Jog S, Sander M, Spies C, Lefrant JY, Backer D De, on behalf of the FENICE Investigators: Fluid challenges in intensive care: the FENICE study: A global inception cohort study. Intensive Care Med 2015; 41:1529–37

3. Woodcock TE, Woodcock TM: Revised Starling equation and the glycocalyx model of transvascular fluid exchange: An improved paradigm for prescribing intravenous fluid therapy 2012; 108:pp 384–94

4. Moore FD, Dagher FJ, Boyden CM, Lee CJ, Lyons JH: Hemorrhage in normal man. I. distribution and dispersal of saline infusions following acute blood loss: clinical kinetics of blood volume support. Ann Surg 1966; 163:485–504

5. Ildikó, László ; Nándor, Öveges ; Zsolt M: Distribution of Crystalloids and Colloids During Fluid Resuscitation: All Fluids Can be Good and Bad? 2017:pp 91–103 at <http://publicatio.bibl.u-szeged.hu/11729/>

6. Adamson RH, Lenz JF, Zhang X, Adamson GN, Weinbaum S, Curry FE: Oncotic pressures opposing filtration across non-fenestrated rat microvessels. J Physiol 2004; 557:889–907

7. Levick JR: Revision of the Starling principle: new views of tissue fluid balance. J Physiol 2004; 557:704

8. Michel C: Starling: the formulation of his hypothesis of microvascular fluid exchange and its significance after 100 years. Exp Physiol 1997; 82:1–30

9. Levick JR, Michel CC: Microvascular fluid exchange and the revised Starling principle. Cardiovasc Res 2010; 87:198–210

10. Ince C: The rationale for microcirculatory guided fluid therapy. Curr Opin Crit Care 2014; 20:301–8

11. Jacob M, Bruegger D, Rehm M, Stoeckelhuber M, Welsch U, Conzen P, Becker BF: The endothelial glycocalyx affords compatibility of Starling's principle and high cardiac interstitial albumin levels. Cardiovasc Res 2007; 73:575–86

12. TP S, Schuerholz T, SP H, Forberger C, MA B, Marx G: High Molecular Weight Hydroxyethyl Starch Solutions Are Not More Effective Than a Low Molecular Weight Hydroxyethyl Starch Solution in a Porcine Model of Septic Shock. Minerva Anestesiol 2013; 79:44–52

13. Kocsi S, Demeter G, Fogas J, Érces D, Kaszaki J, Molnár Z: Central venous oxygen saturation is a good indicator of altered oxygen balance in isovolemic anemia. Acta Anaesthesiol Scand 2012; 56:291–7

14. Ponschab M, Schöchl H, Keibl C, Fischer H, Redl H SC: Preferential effects of low volume versus high volume replacement with crystalloid fluid in a hemorrhagic shock model in pigs. Br Med J Anesthesiol 2015; 15:133

15. Fodor GH, Babik B, Czövek D, Doras C, Balogh ÁL, Bayat S, Habre W, Peták F: Fluid replacement and respiratory function. Eur J Anaesthesiol 2015:1 doi:10.1097/EJA.0000000000000251

16. Reitsma S, Oude Egbrink MGA, Vink H, Berg BM Van Den, Lima Passos V, Engels W, Slaaf DW, Zandvoort MAMJ Van: Endothelial glycocalyx structure in the intact carotid artery: A two-photon laser scanning microscopy study. J Vasc Res 2011; 48:297–306

17. Tánczos K, Molnár Z: Do no harm: use starches? 2013; 79:1101–2

18. Molnár Z, Mikor A, Leiner T, Szakmány T: Fluid resuscitation with colloids of different molecular weight in septic shock. Intensive Care Med 2004; 30:1356–60

19. Finfer S, Bellomo R, Boyce N, French J, Myburgh J, Norton R: A Comparison of Albumin and Saline for Fluid Resuscitation in the Intensive Care Unit. N Engl J Med 2004; 350:2247–56

20. Brunkhorst FM, Engel C, Bloos F, Meier-Hellmann A, Ragaller M, Weiler N, Moerer O, Gruendling M, Oppert M, Grond S, Olthoff D, Jaschinski U, John S,

Rossaint R, Welte T, Schaefer M, Kern P, Kuhnt E, Kiehntopf M, Hartog C, Natanson C, Loeffler M, Reinhart K: Intensive Insulin Therapy and Pentastarch Resuscitation in Severe Sepsis for the German Competence Network Sepsis (SepNet). N Engl J Med 2008; 358:125–39

21. Myburgh JA, Finfer S, Bellomo R, Billot L, Cass A, Gattas D, Glass P, Lipman J, Liu B, McArthur C, McGuinness S, Rajbhandari D, Taylor CB, Webb SAR: Hydroxyethyl starch or saline for fluid resuscitation in intensive care. N Engl J Med 2012; 367:1901–11

22. Perner A, Haase N, Guttormsen AB, Tenhunen J, Klemenzson G, Åneman A, Madsen K, Møller M, Elkjær J, Poulsen L, Bendtsen A, Winding R, Steensen M, Berezowicz P, Winkel P, Wetterslev J: Hydroxyethyl starch 130/0.42 versus Ringer's acetate in severe sepsis. N Engl J Med 2012; 367:124–34

23. Annane D: Effects of Fluid Resuscitation With Colloids vs Crystalloids on Mortality in Critically Ill Patients Presenting With Hypovolemic Shock. Jama 2013:1–9 doi:10.1001/jama.2013.280502

24. Guidet B, Martinet O, Boulain T, Philippart F, Poussel JF, Maizel J: Assessment of hemodynamic efficacy and safety fluid replacement in patients with severe sepsis : The CRYSTMAS study. Crit Care 2012; 16:R94

25. Yates DRA, Davies SJ, Milner HE, Wilson RJT: Crystalloid or colloid for goal-directed fluid therapy in colorectal surgery. Br J Anaesth 2014; 112:281–9

26. Caironi P, Tognoni G, Masson S, Fumagalli R, Pesenti A, Romero M, Fanizza C, Caspani L, Faenza S, Grasselli G, Iapichino G, Antonelli M, Parrini V, Fiore G, Latini R, Gattinoni L, Maggiore Policlinico OP, Sud N, Maria Imbaro SG: Albumin Replacement in Patients with Severe Sepsis or Septic Shock for the ALBIOS Study Investigators*. N Engl J Med 2014; 370:1412–21

27. Benes J, Chytra I, Altmann P, Hluchy M, Kasal E, Svitak R, Pradl R, Stepan M: Intraoperative fluid optimization using stroke volume variation in high risk surgical patients: results of prospective randomized study. Crit Care 2010; 14:R118

28. Lobo DN, Stanga Z, Aloysius MM, Wicks C, Nunes QM, Ingram KL, Risch L,

Allison SP: Effect of volume loading with 1 liter intravenous infusions of 0.9% saline, 4% succinylated gelatine (Gelofusine) and 6% hydroxyethyl starch (Voluven) on blood volume and endocrine responses: a randomized, three-way crossover study in healthy volunteers. Crit Care Med 2010; 38:464–70

29. Eley KA, Duncan Young J, Watt-Smith SR: Epinephrine, norepinephrine, dobutamine, and dopexamine effects on free flap skin blood flow. Plast Reconstr Surg 2012; 130:564–70

30. Rahav Koren R, Suriu C, Yakir O, Akria L, Barhoum M, Braester A: Physicians' lack of knowledge - a possible reason for red blood cell transfusion overuse? Isr J Health Policy Res 2017; 6:1–8

31. Crochemore T, Corrêa TD, Lance MD, Solomon C, Neto AS, Guerra JC de C, Lellis PS, Bernz LM, Nunes N, Mancio CM, Yokoyama APH, Silva E: Thromboelastometry profile in critically ill patients: A single-center, retrospective, observational study. PLoS One 2018; 13:e0192965

32. Salzwedel C, Puig J, Carstens A, Bein B, Molnar Z, Kiss K, Hussain A, Belda J, Kirov MY, Sakka SG, Reuter DA: Perioperative goal-directed hemodynamic therapy based on radial arterial pulse pressure variation and continuous cardiac index trending reduces postoperative complications after major abdominal surgery: a multi-center, prospective, randomized study. Crit Care 2013; 17:R191

33. Sinclair S, James S, Singer M: Intraoperative intravascular volume optimisation and length of hospital stay after repair of proximal femoral fracture: randomised controlled trial. BMJ 1997; 315:909–12

34. Conway DH, Mayall R, Abdul-Latif MS, Gilligan S, Tackaberry C: Randomised controlled trial investigating the influence of intravenous fluid titration using oesophageal Doppler monitoring during bowel surgery. Anaesthesia 2002; 57:845–9

35. Jabaley C, Dudaryk R: Fluid Resuscitation for Trauma Patients: Crystalloids Versus Colloids. Curr Anesthesiol Rep 2014; 4:216–24

36. Qureshi SH, Rizvi SI, Patel NN, Murphy GJ: Meta-analysis of colloids versus

crystalloids in critically ill, trauma and surgical patients. Br J Surg 2015:14–26 doi:10.1002/bjs.9943

37. Bartels K, Thiele RH, Gan TJ: Rational fluid management in today's ICU practice. Crit Care 2013; 17 Suppl 1:S6

38. Lira A, Pinsky MR: Choices in fluid type and volume during resuscitation: impact on patient outcomes. Ann Intensive Care 2014; 4:38

39. Sauaia A, Moore FA, Moore EE, Moser KS, Brennan R, Read RA, Pons PT: Epidemiology of trauma deaths: a reassessment. J Trauma 1995; 38:185–93

40. Acosta JA, Yang JC, Winchell RJ, Simons RK, Fortlage DA, Hollingsworth-Fridlund P, Hoyt DB: Lethal injuries and time to death in a level I trauma center. J Am Coll Surg 1998; 186:528–33

41. Hoyt DB, Bulger EM, Knudson MM, Morris J, Ierardi R, Sugerman HJ, Shackford SR, Landercasper J, Winchell RJ, Jurkovich G: Death in the operating room: an analysis of a multi-center experience. J Trauma 1994; 37:426–32

42. Johansson PI, Sørensen AM, Perner A WK, Wanscher M, Larsen CF, Ostrowski SR: Elderly trauma patients have high circulating noradrenaline levels but attenuated release of adrenaline, platelets, and leukocytes in response to increasing injury severity. Ann Surg 2011; 254:194

43. Johansson PI, Stensballe J, Rasmussen LS, Ostrowski SR: High circulating adrenaline levels at admission predict increased mortality after trauma. J Trauma Acute Care Surg 2012; 72:428–36

44. Nelson A, Berkestedt I, Schmidtchen A, Ljunggren L, Bodelsson M: Increased levels of glycosaminoglycans during septic shock: relation to mortality and the antibacterial actions of plasma. Shock 2008; 30:623–7

45. Marechal X, Favory R, Joulin O, Montaigne D, Hassoun S, Decoster B, Zerimech F, Neviere R: Endothelial glycocalyx damage during endotoxemia coincides with microcirculatory dysfunction and vascular oxidative stress. Shock 2008; 29:572–6

46. Nieuwdorp M, Mooij HL, Kroon J, Atasever B, Spaan JAE, Ince C, Holleman F,

Diamant M, Heine RJ, Hoekstra JBL, Kastelein JJP, Stroes ESG, Vink H: Endothelial glycocalyx damage coincides with microalbuminuria in type 1 diabetes. Diabetes 2006; 55:1127–32

47. Tánczos K, Németh M, Trásy D, László I, Palágyi P, Szabó Z, Varga G, Kaszaki J: Goal-Directed Resuscitation Aiming Cardiac Index Masks Residual Hypovolemia: An Animal Experiment. Biomed Res Int 2015; 2015:1–8

48. Németh M, Tánczos K, Demeter G, Érces D, Kaszaki J, Mikor A, Molnár Z: Central venous oxygen saturation and carbon dioxide gap as resuscitation targets in a hemorrhagic shock. Acta Anaesthesiol Scand 2014; 58:611–9

49. EMANUEL R, BRYANT N, SUZANNE H, JULIE R, ALEXANDRIA M, BERNHARD K, EDWARD P, MICHAEL T, Early Goal-Directed Therapy Collaborative Group: The Ne w E n g l a nd Jour n a l o f Me d ic i ne EARLY GOAL-DIRECTED THERAPY IN THE TREATMENT OF SEVERE SEPSIS AND SEPTIC SHOCK. N Engl J Med 2001; 345:1368–77

50. Pinsky MR: Cardiopulmonary Interactions: Physiologic Basis and Clinical Applications. Ann Am Thorac Soc 2018; 15:S45–8

51. Virág M, Leiner T, Rottler M, Ocskay K, Molnar Z: Individualized Hemodynamic Management in Sepsis. J Pers Med 2021; 11

52. Beest P van, Wietasch G, Scheeren T, Spronk P, Kuiper M: Clinical review: use of venous oxygen saturations as a goal - a yet unfinished puzzle. Crit Care 2011; 15:232

53. Pope J V, Jones AE, Gaieski DF, Arnold RC, Trzeciak S, Shapiro NI, Emergency Medicine Shock Research Network (EMShockNet) Investigators: Multicenter study of central venous oxygen saturation (ScvO(2)) as a predictor of mortality in patients with sepsis. Ann Emerg Med 2010; 55:40-46.e1

54. Gattinoni L, Vasques F, Camporota L, Meessen J, Romitti F, Pasticci I, Duscio E, Vassalli F, Forni LG, Payen D, Cressoni M, Zanella A, Latini R, Quintel M, Marini JJ: Understanding Lactatemia in Human Sepsis. Potential Impact for Early Management. Am J Respir Crit Care Med 2019; 200:582–9

55. Mouncey PR, Power GS, Coats TJ: Early, Goal-Directed Resuscitation for Septic Shock. N Engl J Med 2015; 373:577–8

56. ARISE Investigators, ANZICS Clinical Trials Group, Peake SL, Delaney A, Bailey M, Bellomo R, Cameron PA, Cooper DJ, Higgins AM, Holdgate A, Howe BD, Webb SAR, Williams P: Goal-directed resuscitation for patients with early septic shock. N Engl J Med 2014; 371:1496–506

57. Yealy DM, Kellum JA, Huang DT, Barnato AE, Weissfeld LA, Pike F, Terndrup T, Wang HE, Hou PC, LoVecchio F, Filbin MR, Shapiro NI, Angus DC: A randomized trial of protocol-based care for early septic shock. N Engl J Med 2014; 370:1683–93

58. Lamia B, Monnet X, Teboul JL: Meaning of arterio-venous PCO2 difference in circulatory shock. Minerva Anestesiol 2006; 72:597–604

59. Duhailib Z Al, Hegazy AF, Lalli R, Fiorini K, Priestap F, Iansavichene A, Slessarev M: The Use of Central Venous to Arterial Carbon Dioxide Tension Gap for Outcome Prediction in Critically Ill Patients: A Systematic Review and Meta-Analysis. Crit Care Med 2020; 48:1855–61

60. Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, Kumar A, Sevransky JE, Sprung CL, Nunnally ME, Rochwerg B, Rubenfeld GD, Angus DC, Annane D, Beale RJ, Bellinghan GJ, Bernard GR, Chiche J-D, Coopersmith C, Backer DP De, French CJ, Fujishima S, Gerlach H, Hidalgo JL, Hollenberg SM, Jones AE, Karnad DR, Kleinpell RM, Koh Y, Lisboa TC, et al.: Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. Intensive Care Med 2017; 43:304–77

61. Ferreruela M, Raurich JM, Ayestarán I, Llompart-Pou JA: Hyperlactatemia in ICU patients: Incidence, causes and associated mortality. J Crit Care 2017; 42:200–5

62. Casserly B, Phillips GS, Schorr C, Dellinger RP, Townsend SR, Osborn TM, Reinhart K, Selvakumar N, Levy MM: Lactate measurements in sepsis-induced tissue hypoperfusion: results from the Surviving Sepsis Campaign database. Crit Care Med 2015; 43:567–73

63. Garcia-Alvarez M, Marik P, Bellomo R: Sepsis-associated hyperlactatemia. Crit

Care 2014; 18:503

64. Jhanji S, Vivian-Smith A, Lucena-Amaro S, Watson D, Hinds CJ, Pearse RM: Haemodynamic optimisation improves tissue microvascular flow and oxygenation after major surgery: a randomised controlled trial. Crit Care 2010; 14:R151

65. Sessler DI, Bloomstone JA, Aronson S, Berry C, Gan TJ, Kellum JA, Plumb J, Mythen MG, Grocott MPW, Edwards MR, Miller TE, Perioperative Quality Initiative-3 workgroup, POQI chairs, Miller TE, Mythen MG, Grocott MP, Edwards MR, Physiology group, Preoperative blood pressure group, Intraoperative blood pressure group, Postoperative blood pressure group: Perioperative Quality Initiative consensus statement on intraoperative blood pressure, risk and outcomes for elective surgery. Br J Anaesth 2019; 122:563–74

66. Wesselink EM, Kappen TH, Torn HM, Slooter AJC, Klei WA van: Intraoperative hypotension and the risk of postoperative adverse outcomes: a systematic review. Br J Anaesth 2018; 121:706–21

67. Ackland GL, Brudney CS, Cecconi M, Ince C, Irwin MG, Lacey J, Pinsky MR, Grocott MP, Mythen MG, Edwards MR, Miller TE, Perioperative Quality Initiative-3 workgroup, POQI chairs, Physiology group, Preoperative blood pressure group, Intraoperative blood pressure group, Postoperative blood pressure group: Perioperative Quality Initiative consensus statement on the physiology of arterial blood pressure control in perioperative medicine. Br J Anaesth 2019; 122:542–51

68. Molnar Z, Benes J, Saugel B: Intraoperative hypotension is just the tip of the iceberg: a call for multimodal, individualised, contextualised management of intraoperative cardiovascular dynamics. Br J Anaesth 2020; 125:419–23

69. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, Bellomo R, Bernard GR, Chiche J, Coopersmith CM, Hotchkiss RS, Levy MM, Marshall JC, Martin GS, Opal SM, Rubenfeld GD, Der T Van, Vincent J, Angus DC: The third international consensus definitions for sepsis and septic shock. Jama 2016; 315:801–10

70. Linton RAF, Linton NWF, Kelly F: Is clinical assessment of the circulation reliable

in postoperative cardiac surgical patients? J Cardiothorac Vasc Anesth 2002; 16:4–7

71. Hofmaier F, Dinger K, Braun R, Sterner-Kock A: Range of blood lactate values in farm pigs prior to experimental surgery. Lab Anim 2013; 47:130–2

72. László I, Demeter G, Öveges N, Érces D, Kaszaki J, Tánczos K, Molnár Z: Volume-replacement ratio for crystalloids and colloids during bleeding and resuscitation: an animal experiment. Intensive Care Med Exp 2017; 5:52

73. Marx G, Schindler AW, Mosch C, Albers J, Bauer M, Gnass I, Hobohm C, Janssens U, Kluge S, Kranke P, Maurer T, Merz W, Neugebauer E, Quintel M, Senninger N, Trampisch H-J, Waydhas C, Wildenauer R, Zacharowski K, Eikermann M: Intravascular volume therapy in adults: Guidelines from the Association of the Scientific Medical Societies in Germany. Eur J Anaesthesiol 2016; 33:488–521

74. Trásy D, Tánczos K, Németh M, Hankovszky P, Lovas A, Mikor A, László I, Hajdú E, Osztroluczki A, Fazakas J, Molnár Z: Early procalcitonin kinetics and appropriateness of empirical antimicrobial therapy in critically ill patients. A prospective observational study. J Crit Care 2016; 34:50–5

75. Hahn RG, Lyons G: The half-life of infusion fluids: An educational review. Eur J Anaesthesiol 2016; 33:475–82

76. Ewaldsson C-A, Hahn RG: Kinetics and extravascular retention of acetated ringer's solution during isoflurane or propofol anesthesia for thyroid surgery. Anesthesiology 2005; 103:460–9

77. Hahn RG: Crystalloids should be second choice for goal-directed fluid therapy. Eur J Anaesthesiol 2020; 37:414–5

78. László I, Janovszky Á, Szabó A, Molnár Z: Reply to: crystalloids should be second choice for goal-directed fluid therapy. Eur J Anaesthesiol 2020; 37:415–6

79. Bone RC, Fisher CJ, Clemmer TP, Slotman GJ, Metz CA, Balk RA: A Controlled Clinical Trial of High-Dose Methylprednisolone in the Treatment of Severe Sepsis and Septic Shock. N Engl J Med 1987; 317:653–8

80. Giordano G, Pugliese F, Bilotta F: Effects of goal-directed crystalloid vs. colloid fluid therapy on microcirculation during free flap surgery. Eur J Anaesthesiol 2020; 37:413

81. László I, Janovszky Á, Szabó A, Molnár Z: Reply to: effects of goal-directed crystalloid vs. colloid fluid therapy on microcirculation during free flap surgery. Eur J Anaesthesiol 2020; 37:413–4

82. Lee WL, Slutsky AS: Sepsis and endothelial permeability. N Engl J Med 2010; 363:689–91

83. Kolářová H, Ambrůzová B, Svihálková Šindlerová L, Klinke A, Kubala L: Modulation of endothelial glycocalyx structure under inflammatory conditions. Mediators Inflamm 2014; 2014:694312

84. Honore PM, Jamez J, Wauthier M, Lee PA, Dugernier T, Pirenne B, Hanique G, Matson JR: Prospective evaluation of short-term, high-volume isovolemic hemofiltration on the hemodynamic course and outcome in patients with intractable circulatory failure resulting from septic shock. Crit Care Med 2000; 28:3581–7

85. Ikeda M, Matsumoto H, Ogura H, Hirose T, Shimizu K, Yamamoto K, Maruyama I, Shimazu T: Circulating syndecan-1 predicts the development of disseminated intravascular coagulation in patients with sepsis. J Crit Care 2018; 43:48–53

86. Reitsma S, Slaaf DW, Vink H, Zandvoort MAMJ Van, Oude Egbrink MGA: The endothelial glycocalyx: Composition, functions, and visualization. Pflugers Arch Eur J Physiol 2007; 454:345–59

87. Johansson PI, Stensballe J, Rasmussen LS, Ostrowski SR: A High Admission Syndecan-1 Level, A Marker of Endothelial Glycocalyx Degradation, Is Associated With Inflammation, Protein C Depletion, Fibrinolysis, and Increased Mortality in Trauma Patients. Ann Surg 2011; 254:194–200

88. Nelson A, Johansson J, Tyden J, Bodelsson M: Circulating syndecans during critical illness. APMIS 2017; 125:468–75

89. Uzawa K, Ushiyama A, Mitsuda S, Ando T, Sawa M, Miyao H, Yorozu T: The

protective effect of hydroxyethyl starch solution on the glycocalyx layer in an acute hemorrhage mouse model. J Anesth 2020; 34:36–46

90. László I, Csákány L, Veres A, Németh M, Poles M, Kaszaki J, Molnár Z, Szabó A: Az endotheliális glycocalyx megjelenítése és vastagságának meghatározása intravitális mikroszkópiával. ANESZTEZIOLÓGIA ÉS INTENZÍV TERÁPIA 2021; 51:11–20

91. Okada H, Takemura G, Suzuki K, Oda K, Takada C, Hotta Y, Miyazaki N, Tsujimoto A, Muraki I, Ando Y, Zaikokuji R, Matsumoto A, Kitagaki H, Tamaoki Y, Usui T, Doi T, Yoshida T, Yoshida S, Ushikoshi H, Toyoda I, Ogura S: Three-dimensional ultrastructure of capillary endothelial glycocalyx under normal and experimental endotoxemic conditions. Crit Care 2017; 21:261

92. Bongoni AK, Lu B, McRae JL, Salvaris EJ, Toonen EJM, Vikstrom I, Baz Morelli A, Pearse MJ, Cowan PJ: Complement-mediated Damage to the Glycocalyx Plays a Role in Renal Ischemia-reperfusion Injury in Mice. Transplant direct 2019; 5:e341

93. Luker JN, Vigiola Cruz M, Carney BC, Day A, Moffatt LT, Johnson LS, Shupp JW: Shedding of the endothelial glycocalyx is quantitatively proportional to burn injury severity. Ann Burns Fire Disasters 2018; 31:17–22

94. Yu W-Q, Zhang S-Y, Fu S-Q, Fu Q-H, Lu W-N, Zhang J, Liang Z-Y, Zhang Y, Liang T-B: Dexamethasone protects the glycocalyx on the kidney microvascular endothelium during severe acute pancreatitis. J Zhejiang Univ Sci B 2019; 20:355–62

95. Martin L, Gombert A, Chen J, Liebens J, Verleger J, Kalder J, Marx G, Jacobs M, Thiemermann C, Schuerholz T: The beta-d-Endoglucuronidase Heparanase Is a Danger Molecule That Drives Systemic Inflammation and Correlates with Clinical Course after Open and Endovascular Thoracoabdominal Aortic Aneurysm Repair: Lessons Learnt from Mice and Men. Front Immunol 2017; 8:681

96. Dane MJC, Khairoun M, Lee DH, Berg BM van den, Eskens BJM, Boels MGS, Teeffelen JWGE van, Rops ALWMM, Vlag J van der, Zonneveld AJ van, Reinders MEJ, Vink H, Rabelink TJ: Association of kidney function with changes in the

endothelial surface layer. Clin J Am Soc Nephrol 2014; 9:698–704

97. Ince C: Hemodynamic coherence and the rationale for monitoring the microcirculation. Crit Care 2015; 19:S8

98. Kimberger O, Arnberger M, Brandt S, Plock J, Sigurdsson GH, Kurz A, Hiltebrand L: Goal-directed Colloid Administration Improves the Microcirculation of Healthy and Perianastomotic Colon. Anesthesiology 2009; 110:496–504

99. Landsverk SA, Kvandal P, Kjelstrup T, Benko U, Bernjak A, Stefanovska A, Kvernmo H, Kirkeboen KA: Human skin microcirculation after brachial plexus block evaluated by wavelet transform of the laser Doppler flowmetry signal. Anesthesiology 2006; 105:478–84

100. Rossi M, Carpi A, Galetta F, Franzoni F, Santoro G: The investigation of skin blood flowmotion: a new approach to study the microcirculatory impairment in vascular diseases? Biomed Pharmacother 2006; 60:437–42

101. Wang WZ, Anderson G, Fleming JT, Peter FW, Franken RJ, Acland RD, Barker J: Lack of nitric oxide contributes to vasospasm during ischemia/reperfusion injury. Plast Reconstr Surg 1997; 99:1099–108

102. Gazdag A, Chen L, Hagen PO, Seaber A V, Urbaniak JR: Effect of denervation on endothelium-derived relaxing factor-dependent relaxation in the rat cremaster muscle. Microsurgery 1993; 14:494–500

103. László I, Janovszky Á, Lovas A, Vargán V, Öveges N, Tánczos T, Mikor A, Trásy D, Lóderer Z, Piffkó J, Szabó A, Molnár Z: Effects of goal-directed crystalloid vs. colloid fluid therapy on microcirculation during free flap surgery: A randomised clinical trial. Eur J Anaesthesiol 2019; 36:592–604

104. Monnet X, Vaquer S, Anguel N, Jozwiak M, Cipriani F, Richard C, Teboul J-L: Comparison of pulse contour analysis by Pulsioflex and Vigileo to measure and track changes of cardiac output in critically ill patients. Br J Anaesth 2015; 114:235–43

105. Grensemann J, Defosse JM, Wieland C, Wild UW, Wappler F, Sakka SG: Comparison of PulsioFlex® uncalibrated pulse contour method and a modified Fick

principle with transpulmonary thermodilution measurements in critically ill patients. Anaesth Intensive Care 2016; 44:484–90

APPENDIX