Best Practice & Research Clinical Anaesthesiology 28 (2014) 227–234

Contents lists available at ScienceDirect

Best Practice & Research Clinical Anaesthesiology

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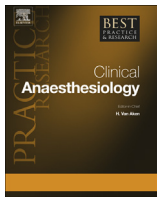

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# Role of the glycocalyx in fluid management: Small things matter☆

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*Keywords:*
colloids
crystalloids
endothelial glycocalyx
endothelial surface layer
fluid therapy

Intravenous fluid therapy and perception of volume effects are often misunderstood. The pharmacokinetical difference between colloids and crystalloids depends on the condition of the vascular permeability barrier. Its functioning is still largely based on Starling's principle from 1896, realising that transport of fluid to and from the interstitial space follows the balance between opposing oncotic and hydrostatic pressures. In the past decade, the endothelial glycocalyx, located on the luminal side of healthy vasculature, has increasingly been taken into consideration around models of transvascular fluid filtration. While crystalloids can freely pass through the glycocalyx, colloids are held back in the vasculature by this structure. This is reflected by a markedly higher intravascular persistence of isooncotic colloids (80–100%) versus crystalloids (around 20%), at least as long as the glycocalyx is intact. Protecting this structure in surgical practice means limiting the surgical trauma and avoiding intravascular hypervolemia.


Over the past years, fluid management has been a controversially discussed topic [1]. Meanwhile, consensus has been achieved to the effect that the composition of crystalloids should resemble plasma concentrations of electrolytes and, therefore, balanced solutions should be preferred over isotonic

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http://dx.doi.org/10.1016/j.bpa.2014.06.003
1521-6896/

saline [2]. Beyond that, intravascular hypo- and hypervolemia are regarded equally dangerous and should both be avoided [3]. The question when to use colloids or crystalloids, however, remains debatable. Unfortunately, this emotional debate still largely suffers from a lack of rationality, often ignoring important and well-established scientific facts [4]: First, infusion solutions are quite obviously drugs with indications, contraindications, and side effects. Therefore, the current "safety" discussion is erroneous, ignoring that the application of any potent drug has always to be the result of carefully outweighing the pros and cons on an individual basis. Second, colloids and crystalloids are completely different classes of drugs with different pharmacokinetics and target compartments. The reasons for this are the physiological properties of the vascular permeability barrier, being made up of not only the endothelial cell line but also a structure that has only recently moved into the centre of attention beyond: the endothelial surface layer (ESL), consisting of a fragile glycocalyx skeleton and bound plasma constituents [5].

This article will combine old and new physiological knowledge to the related clinical effects on pharmacokinetics of intravenous (IV) fluids. In spite, or maybe even because, of not discussing current trials and outcome-based evidence, it provides crystal–clear, largely unquestioned, and important knowledge for clinical practice.

## The perioperative fluid and volume loss – from fairy tales to real problems

Surgery and trauma have traditionally been believed to cause an impressive primary loss out of the intravascular space, even without massive blood losses. This assumed problem was, for decades, treated with high basal crystalloidal maintenance rates to, nonetheless, maintain cardiac preload. In fact, fluid handling by generations of anaesthesiologists during major surgery in humans has led to an excess of several litres in the measurable sensible perioperative fluid balance, that is, measurable input (infusions and transfusions) minus measurable output (blood loss and urine production). This was traditionally interpreted to be the necessary therapeutical answer to three types of unmeasurable losses in clinical routine: the preoperative deficit, the insensible perspiration and third-space shifting triggered by surgery and trauma [1]. However, several trials showed a related perioperative body weight gain of up to 10 kg, suggesting that evaporation might not play a leading role in this context [1]. Indeed, Lamke et al. demonstrated the contribution of the insensible perspiration to perioperative fluid needs to be much smaller than assumed [6]. Additionally, the actual impact of fasting on intravascular volume state should be limited: Even after 10 h of fasting, intravascular blood volume was shown to be within normal ranges [7]. Above that, the recommended preoperative period of stopped oral intake of clear liquids is decreasing more and more. As guidelines are currently recommending 2 h for clear liquids, at least today there should be no relevant contribution to a preoperative deficit. Detailed searches for the mysterious third space revealed that this presumably fluid-consuming compartment actually does not exist [8].

Most likely, perioperative losses of intravascular volume outside acute bleeding is nothing more and nothing less than a simple shift within the extracellular compartment, towards the interstitial space. The related pathophysiology is not difficult, but, nevertheless, exciting and of impact for clinical fluid and volume handling if the target is preload stabilisation and oedema prevention.

### *Physiology I – the classical view to compartments and barriers*

In healthy humans, approximately 60% of total body mass is made up by water (approximately 45 l in an 80-kg healthy male adult). Intracellular fluid comprises 30 l of the body water which is separated from the extracellular compartment containing the remaining 15 l by the cell membrane (Fig. 1) [9]. The latter is practically impermeable for all small and large solutes like electrolytes, proteins, colloids, etc. Nevertheless, it is not able to remain in shape in the context of hydrostatic gradients which immediately lead to cell shrinking or cell swelling and, depending on the affected tissue, to organ failure. The extracellular compartment (15 l) consists of the plasma volume (1/5, i.e., about 3 l) and the interstitial space (4/5, i.e., about 12 l). Beyond that, there are negligible amounts of so-called trans-cellular fluids, such as gastrointestinal secretion and cerebrospinal and ocular fluid which should not be discussed here. The two sub-compartments of the extracellular space are anatomically separated by

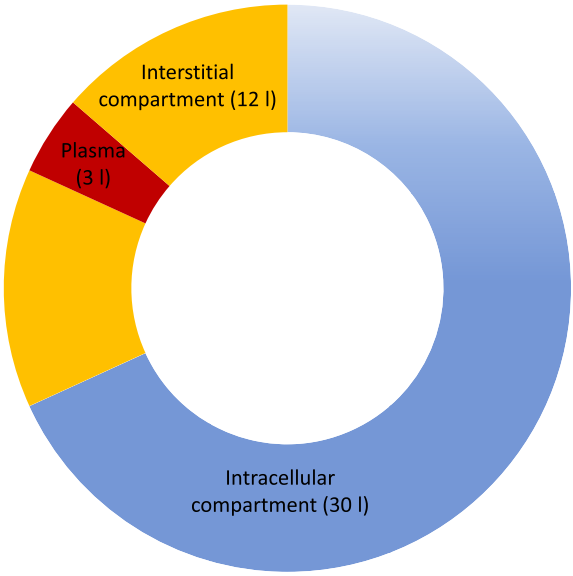


**Fig. 1.** Distribution of the body water in a cardiocirculatory-compensated, normal-weighed male adult (average values). Extracellular compartment (15 l) = plasma + interstitial compartment (taken from Jacob and Chappell [9]).

the vascular barrier. In contrast to the cell membrane, it allows free and easy exchange of small molecules such as electrolytes, together with the accompanying water load, while large molecules like proteins and colloids are held back sufficiently as long as it is functionally intact. This enables the circulation to generate a positive intravascular blood pressure without unlimited loss of plasma water towards the interstitial space.

The British physiologist Ernest Starling introduced his famous physiological model of the vascular barrier as early as in 1896 [10]: Inside the blood vessels, both the hydrostatic pressure and the colloid osmotic pressure are high. By contrast, Starling believed the interstitial space to contain only a very low concentration of proteins and to have a low hydrostatic pressure. This theoretically should result in two opposing forces, leading in a net filtration per unit of time in the high-pressure segments which should be compensated, to a large part, by reabsorption at the venular aspect, any fluid excess being drained by the lymphatic system. According to this classical principle, a sufficient colloid osmotic pressure within the circulatory space and an extremely low one outside appears necessary to provide a physiologically active inward-directed force in order to successfully oppose the hydrostatic pressure gradient. However, several experimental setups have shown that the classical principle as described by Starling might be inaccurate. Ten years ago, Adamson and coworkers showed by their isolated rat mesenteric microvessel model that the barrier also works if the oncotic pressure outside the endothelial cell line equals the intravascular one [11]. This has been confirmed by isolated organ models and by the immunohistochemical observation that the interstitial space, in fact, is loaded with plasma proteins even in the cardiocirculatory steady state [12]. Beyond that, the theory of venular reabsorption of plasma ultrafiltrate meanwhile has increasingly been challenged [5]. Obviously, things around vascular barrier competence are not as easy as suggested more than 100 years ago. The classical Starling model needed an update.

*Physiology II – current concepts on vascular barrier competence*

The past decade brought exciting new insights into the physiology of the vascular barrier. Obviously, not the endothelial cell barrier, but a small, fragile, at first sight insignificant structure at the luminal endothelial surface determines what we call the vascular barrier competence. This increasingly led to a model of vascular functioning fitting much better into the newer physiological observations of the past two decades than the classical view. The so-called "endothelial glycocalyx," a carbohydrate-rich layer

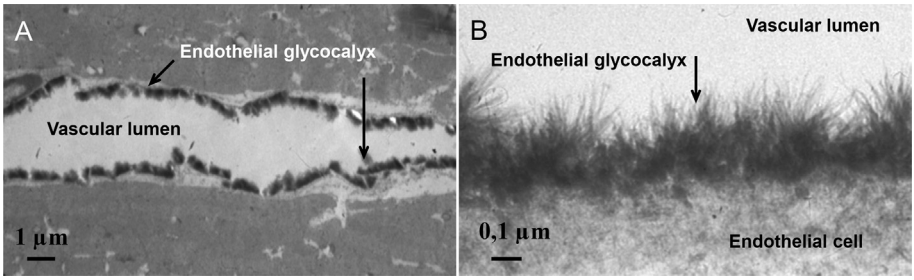


**Fig. 2.** Electron microscopic pictures showing an intact endothelial glycocalyx in coronary vessels of a guinea pig heart (taken from Chappell et al. [30]).

coating the luminal side of the healthy vascular endothelium, is constituted of membrane-bound proteoglycans and glycoproteins, mainly syndecan and glypican, carrying negatively charged side chains like heparan-, dermatan-, and chondroitin sulfates (Fig. 2) [5]. Hyaluronic acid is another important part of this structure. Together with bound plasma proteins, mainly human albumin, it builds up to the physiologically active ESL with a functional thickness of over 1 µm, quantitatively fixing around 700–1000 ml of plasma at the endothelial surface under normal conditions [13]. The plasma molecules of this noncirculating part are in a dynamic equilibrium with those of the circulating plasma volume, representing the distribution space of the circulating blood cells. The physiological role of the ESL is impressive: Obviously, within the high-pressure segment the endothelial glycocalyx acts as an intravascular fringe, retaining plasma constituents that are hydrostatically forced towards the interstitials space and building up the ESL. A small remaining flux of plasma ultrafiltrate through small breaks within the intercellular junction strand permanently cleans a protein-free molecular space below this layer towards the interstitial space from where it is permanently removed by the lymphatic system. Therefore, according to current knowledge, the inward-directed oncotic force keeping the vascular compartment in balance develops exclusively at the luminal side of the anatomical vessel wall, across the endothelial glycocalyx, having nothing to do with the interstitial protein load. In the low-pressure venular segments, the vascular barrier competence might be of less significance and most likely does not necessitate nor allow net reabsorption [5].

Beyond its significance for vascular barrier, the ESL participates in various physiological processes such as preventing firm adhesion of leukocytes and blood platelets to the vessel wall, transmission of shear stress, and in modulation of inflammatory and haemostatic processes [5,14,15].

The knowledge about the two required components of a properly working vascular barrier, an intact endothelial glycocalyx, and a sufficient minimal concentration of suitable plasma proteins building up to the ESL is important for the clinician, as it gives a good estimation of what might happen if this skeleton structure is altered. Pathophysiological sequelae of glycocalyx failure or perturbation include generation of tissue oedema, systemic inflammatory response syndrome, diabetic angiopathy, and, possibly, atherogenesis. Situations in which damage to the glycocalyx has been reported include, for example, ischemia/reperfusion, sepsis, volume overload, diabetes, and trauma [16–20]. Concerning the perioperative situation and critical illness in general, ischemia/reperfusion injury and inflammation appear interesting. Concerning fluid and volume handling, especially the aspect of intravascular hypervolemia, most likely threatening the integrity of the endothelial glycocalyx via the release of natriuretic peptides from the atria activating various metalloproteinases, deserves attention [19,21].

The classical Starling principle needed to be modified into a modern form. Obviously, the integrity of the endothelial glycocalyx plays a major role in vascular integrity, fluid shifting, and the different behaviours of infusion solutions.

## Distribution behaviour of crystalloids and colloids in clinical practice

The target of fluid and volume handling in perioperative and ICU practice is to maintain or restore the normal qualitative and quantitative composition of all compartments, presuming that this might be

of advantage for the patient. In the context of acute bleeding, volume therapy in the strict sense of the word normally aims at maintaining blood volume, being the quantitative component of cardiac preload, on top of maintaining the extracellular fluid compartment and adequate vasotension. While the use of crystalloids to replace ongoing extracellular losses via urinary output and insensible perspiration is largely unquestioned in clinical practice, the debate whether crystalloids or colloids should be used to deal with acute blood losses beyond remains controversial; it is currently not possible to answer this question by reliable outcome data. Nevertheless, it appears reasonable that a chosen strategy should aim at rapidly restoring blood volume and preventing interstitial oedema as long and as far as possible. In the centre of attention, when evaluating crystalloids and colloids in this context, is, therefore, the intravascular volume effect. The latter is defined as that part remaining intravascularly after intravenous infusion, not being directly excreted via a renal first-pass effect or shifted towards the tissues. In other words: it is that part of a volume replacement drug working properly. Clinical data generated by a group from the University Hospital of Munich indicated a completely different distribution behaviour of crystalloids and colloids when used for volume replacement in humans which is very close to the physiological expectations, at least as long as the ESL appears to be intact [23,24]. The chosen clinical model was acute normovolemic haemodilution (ANH), simulating the bleeding patient who simultaneously receives crystalloids or colloids in order to maintain blood volume. This was combined with direct blood volume measurements applying a nonradioactive double-tracer-approach using indocyanine green to trace plasma volume and fluorescein-labelled autologous erythrocytes to assess red cell volume before and after the hemodilution procedure as described and validated previously [25]. With these data, the intravascular persistence of the respectively tested volume replacement preparation can be measured with the highest precision currently described in the literature (Fig. 3) [19,23,26].

Using a crystalloid (lactated Ringer's) for volume replacement in order to treat acute bleeding led to a severe breakdown of blood volume [24]. From the circulation of 10 cardiopulmonary healthy patients scheduled for major abdominal surgery, 1097 ± 285 ml of blood was withdrawn during approximately 30 min and simultaneously replaced by the threefold amount of crystalloid (3430 ± 806 ml). This led to a significant and clinically relevant decrease in blood volume by >10% (from 3959 ± 387 ml to 3501 ± 499 ml), the volume effect of lactated Ringer's having been measured with a poor 17 ± 10% [24]. If an isooncotic colloid was used to deal with a comparable problem in a comparable collective, by contrast, the result was a completely different one: acute controlled withdrawal of 1431 ± 388 ml of blood was treated online with the infusion of 1686 ± 437 ml of 6% hydroxyethyl starch 130/0.4 ($n = 10$) [26]. This led to a stabilisation of blood volume at a high normal level, having slightly increased from 4142 ± 986 to 4360 ± 1083 ml. The calculated volume effect of this isooncotic volume replacement preparation was 98 ± 12% and stable for at least 60 min. A comparable volume effect under these conditions was confirmed for 6% HES 200/0.5 (90 ± 18%) and 5% human albumin (87 ± 14%) [23,27]. Obviously, crystalloids and colloids used for acute volume replacement fulfil exactly the expectation of Ernest Starling who suggested >100 years ago a distribution of crystalloids, within the extracellular compartment, to be 80% towards the interstitial space, while large molecules (e.g., proteins) and the accompanying water load should be retained at the properly working vascular barrier [10].

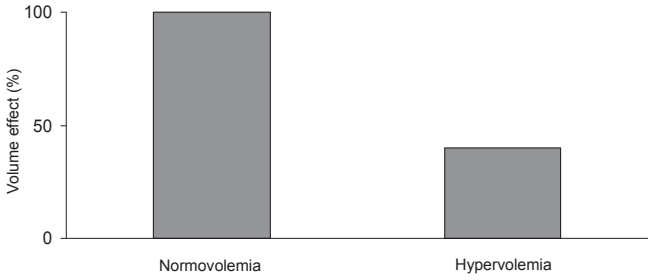


**Fig. 3.** The context sensitivity of volume effects of isooncotic colloids: while 6% hydroxethylstarch or 5% human albumin remain within the circulation to almost 100% if infused as a substitute during acute blood loss (left-hand column) [23], the preparations vanish out of the vasculature to a large extent if applied as hypervolemic bolus (right-hand column) [19] (taken from Jacob et al. [8]).

In the case of a deficit of blood volume, isooncotic preparations of albumin and hydroxyethyl starch appear to be much more volume effective than isotonic crystalloids.

The high intravascular volume effect of isooncotic colloids, however, decreases dramatically if they are infused outside a proper indication, that is, into a normovolemic and, therefore, stable circulation as seen in a further group of patients. Again, 10 patients in a steady state were treated with a colloidal bolus (1379 ± 128 ml of 5% human albumin), but without simultaneous blood withdrawal [19]. This hypervolemic hemodilution was able to somewhat expand blood volume, but by far not at the infused amount (from 4189 ± 769 ml to 4713 ± 868 ml), the volume effect of 5% human albumin having been now, outside a proper indication for volume replacement therapy, only 38 ± 21%. This was also confirmed for 6% HES 200/0.5 which showed under comparable conditions an acute intravascular persistence of poor 43 ± 26% [19]. This effect was accompanied by a severe breakdown of the total volume of the noncirculating plasma volume in these patients towards 1/3 of the initial value, most likely due to the release of natriuretic peptides from the atria which might activate various metalloproteinases that digest the endothelial glycocalyx as mentioned before [21,22]. Therefore, the poor volume effect of colloids outside their primary indication "intravascular hypovolemia" has to be interpreted as the clinical correlate of a biological catastrophe occurring at the endothelial surface: the destruction of the endothelial glycocalyx, the core structure of vascular barrier competence.

Colloids only work properly if they are indicated, and the only indication is intravascular hypovolemia. Using them to load a normovolemic circulation destroys the ESL, leading to vascular barrier insufficiency. This might be the explanation for some negative effects and low-volume effects seen in recent randomised controlled trials, evaluating colloid use in hemodynamically stable patients and, therefore, outside their indication [28,29].

## Current concepts on fluid shifting towards the interstitial space

The increasing knowledge about existence and significance of the ESL requires the formal differentiation between two types of shifting with completely different pathophysiologies related to fluid and volume handling: Type 1 is not a pathophysiological surprise; this physiological, almost colloid-free shift of fluid and electrolytes out of the vasculature principally appears, to a small extent, all the time as a result of an outweighing net hydrostatic gradient towards the tissues [1]. Under normal conditions, it is managed by the lymphatic system. When quantitatively overwhelming its capacity, it leads to interstitial oedema, perioperatively often seen as the inevitable, predictable, and constant consequence of the therapeutical concept to use crystalloids to deal with acute blood losses beyond maintenance. The integrity of the vascular barrier does not matter in this context, as even when being intact it is not able to retain electrolytes which always distribute evenly across the whole extracellular space. Therefore, the resulting interstitial oedema should be, at least in principle, resolvable. Type 2, by contrast, is a pathological protein-rich plasma shift towards the tissues. Accordingly, this type of interstitial oedema is the clinical correlate of a substantial and lasting alteration of a core structure of the vascular barrier, mainly due to surgery, trauma, inflammation, and intravascular hypervolemia. This problem should most likely be avoided as far as possible in the perioperative period, a good approach being a quick and atraumatic surgical technique as a first-line measure, which has to be supported by the avoidance of intravascular hypervolemia as far as possible.

Interstitial oedema is a relevant and increasingly acknowledged clinical problem, endangering patient outcome. In elective surgery, its incidence seems to be closely related to perioperative fluid behaviour.

## Fluid and volume handling in clinical practice

Crystalloids and colloids do have completely different distribution behaviours: Crystalloids target at the whole extracellular space. Therefore, it is reasonable to use them to replace ongoing extracellular losses the body knows also from daily life outside surgery and critical illness like urinary output and insensible perspiration [1]. If, beyond that, the compartments are confronted with acute bleeding below transfusion triggers, it appears reasonable to answer this primarily isolated intravascular problem with a class of drugs targeting at the same compartment. This accounts for isooncotic

preparations of human albumin and hydroxyethyl starch; the decision has to be made on an individual basis, taking not only the nature of the acute problem and preexisting illnesses of the patient but also economic aspects into account. Prophylactic hypervolemia in order to have an intravascular safety margin for possible later bleeding or vasodilation has to be considered outdated in the elective surgical situation, as it breaks down the vascular barrier, decreasing cardiac preload and causing interstitial oedema. There are good hints in the literature that these conditions severely decrease overall outcome [1].

**Practice points**

- The endothelial surface layer consists of the endothelial glycocalyx and bound plasma constituents, mainly albumin
- The endothelial surface layer is a crucial part of the vascular barrier
- Crystalloids and colloids have different distribution compartments
- An intact glycocalyx is a prerequisite for high-volume effects of colloids
- Perturbation of the glycocalyx causes a breakdown of barrier functioning

**Research agenda**

- Large randomised controlled trials evaluating the effect of colloids in the elective patient during major surgery are necessary
- Safety trials for the use of artificial colloids in surgery and trauma are needed
- Effects and pathophysiological insights of hypervolemia on glycocalyx integrity need to be evaluated

## Funding

No funding was received for preparing and writing this manuscript.

## Conflicts of interest

DC has held lectures for BBraun and Fresenius Kabi and received a research grant from Grifols.

MJ has received research grants and has lectures from Baxter, BBraun, CSL Behring, Fresenius Kabi, Grifols, and Serumwerke Bernburg. MJ is on the Albumin Advisory Board of Grifols.

## References

*[1] Chappell D, Jacob M, Hofmann-Kiefer K, et al. A rational approach to perioperative fluid management. Anesthesiology 2008;109:723–40.
*[2] Shaw AD, Bagshaw SM, Goldstein SL, et al. Major complications, mortality, and resource utilization after open abdominal surgery: 0.9% saline compared to plasma-lyte. Ann Surg 2012;255:821–9.
*[3] Myburgh JA, Mythen MG. Resuscitation fluids. N Engl J Med 2013;369:1243–51.
[4] Chappell D, Jacob M. Hydroxyethyl starch – the importance of being earnest. Scand J Trauma Resusc Emerg Med 2013;21:61.
*[5] Becker BF, Chappell D, Jacob M. Endothelial glycocalyx and coronary vascular permeability: the fringe benefit. Basic Res Cardiol 2010;105:687–701.
[6] Lamke LO, Nilsson GE, Reithner HL. Insensible perspiration from the skin under standardized environmental conditions. Scand J Clin Lab Invest 1977;37:325–31.
[7] Jacob M, Chappell D, Conzen P, et al. Blood volume is normal after pre-operative overnight fasting. Acta Anaesthesiol Scand 2008;52:522–9.

*[8] Jacob M, Chappell D, Rehm M. The 'third space'—fact or fiction? Best Pract Res Clin Anaesthesiol 2009;23:145–57.
[9] Jacob M, Chappell D. Effects of perioperative fasting on haemodynamics and intravascular volumes. Best Pract Res Clin Anaesthesiol 2012;26:421–30.
[10] Starling EH. On the absorption of fluids from the connective tissue spaces. J Physiol 1896;19:312–26.
*[11] Adamson RH, Lenz JF, Zhang X, et al. Oncotic pressures opposing filtration across non-fenestrated rat microvessels. J Physiol 2004;557:889–907.
[12] Jacob M, Bruegger D, Rehm M, et al. The endothelial glycocalyx affords compatibility of Starling's principle and high cardiac interstitial albumin levels. Cardiovasc Res 2007;73:575–86.
*[13] Pries AR, Secomb TW, Gaehtgens P. The endothelial surface layer. Pflugers Archiv Eur J Physiol 2000;440:653–66.
[14] Chappell D, Dorfler N, Jacob M, et al. Glycocalyx protection reduces leukocyte adhesion after ischemia/reperfusion. Shock 2010;34:133–9.
[15] Jacob M, Rehm M, Loetsch M, et al. The endothelial glycocalyx prefers albumin for evoking shear stress-induced, nitric oxide-mediated coronary dilatation. J Vasc Res 2007;44:435–43.
*[16] Nelson A, Berkestedt I, Schmidtchen A, et al. Increased levels of glycosaminoglycans during septic shock: relation to mortality and the antibacterial actions of plasma. Shock 2008;30:623–7.
[17] Rehm M, Bruegger D, Christ F, et al. Shedding of the endothelial glycocalyx in patients undergoing major vascular surgery with global and regional ischemia. Circulation 2007;116:1896–906.
[18] Nieuwdorp M, van Haeften TW, Gouverneur MC, et al. Loss of endothelial glycocalyx during acute hyperglycemia coincides with endothelial dysfunction and coagulation activation in vivo. Diabetes 2006;55:480–6.
*[19] Rehm M, Haller M, Orth V, et al. Changes in blood volume and hematocrit during acute preoperative volume loading with 5% albumin or 6% hetastarch solutions in patients before radical hysterectomy. Anesthesiology 2001;95:849–56.
[20] Johansson PI, Stensballe J, Rasmussen LS, et al. 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.
[21] Bruegger D, Schwartz L, Chappell D, et al. Release of atrial natriuretic peptide precedes shedding of the endothelial glycocalyx equally in patients undergoing on- and off-pump coronary artery bypass surgery. Basic Res Cardiol 2011;106:1111–21.
[22] Volta CA, Alvisi V, Campi M, et al. Influence of different strategies of volume replacement on the activity of matrix metalloproteinases: an in vitro and in vivo study. Anesthesiology 2007;106:85–91.
*[23] Rehm M, Orth V, Kreimeier U, et al. Changes in intravascular volume during acute normovolemic hemodilution and intraoperative retransfusion in patients with radical hysterectomy. Anesthesiology 2000;92:657–64.
[24] Jacob M, Chappell D, Hofmann-Kiefer K, et al. The intravascular volume effect of Ringer's lactate is below 20%: a prospective study in humans. Crit Care 2012;16:R86.
[25] Haller M, Akbulut C, Brechtelsbauer H, et al. Determination of plasma volume with indocyanine green in man. Life Sci 1993;53:1597–604.
[26] Jacob M, Rehm M, Orth V, et al. Exact measurement of the volume effect of 6% hydoxyethyl starch 130/0.4 (Voluven) during acute preoperative normovolemic hemodilution. Anaesthesist 2003;52:896–904.
[27] Rehm M, Orth VH, Kreimeier U, et al. Changes in blood volume during acute normovolemic hemodilution with 5% albumin or 6% hydroxyethylstarch and intraoperative retransfusion. Anaesthesist 2001;50:569–79.
[28] Perner A, Haase N, Guttormsen AB, et al. Hydroxyethyl starch 130/0.42 versus Ringer's acetate in severe sepsis. N Engl J Med 2012;367:124–34.
[29] Brunkhorst FM, Engel C, Bloos F, et al. Intensive insulin therapy and pentastarch resuscitation in severe sepsis. N Engl J Med 2008;358:125–39.
[30] Chappell D, Jacob M, Hofmann-Kiefer K, et al. Hydrocortisone preserves the vascular barrier by protecting the endothelial glycocalyx. Anesthesiology 2007;107:776–84.