Isoflurane in Contrast to Propofol Promotes Fluid Extravasation during Cardiopulmonary Bypass in Pigs

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ABSTRACT

Background: A highly positive intraoperative fluid balance should be prevented as it negatively impacts patient outcome. Analysis of volume-kinetics has identified an increase in interstitial fluid volume after crystalloid fluid loading during isoflurane anesthesia. Isoflurane has also been associated with postoperative hypoxemia and may be associated with an increase in alveolar epithelial permeability, edema formation, and hindered oxygen exchange. In this article, the authors compare fluid extravasation rates before and during cardiopulmonary bypass (CPB) with isoflurane- *versus* propofol-based anesthesia.

Methods: Fourteen pigs underwent 2 h of tepid CPB with propofol (P-group; n = 7) or isoflurane anesthesia (I-group; n = 7). Fluid requirements, plasma volume, colloid osmotic pressures in plasma and interstitial fluid, hematocrit levels, and total tissue water content were recorded, and fluid extravasation rates calculated.

Results: Fluid extravasation rates increased in the I-group from the pre-CPB level of 0.27 (0.13) to 0.92 (0.36) $ml \cdot kg^{-1} \cdot min^{-1}$, but remained essentially unchanged in the P-group with significant between-group differences during CPB ($p_b = 0.002$). The results are supported by corresponding changes in interstitial colloid osmotic pressure and total tissue water content.

Conclusions: During CPB, isoflurane, in contrast to propofol, significantly contributes to a general increase in fluid shifts from the intravascular to the interstitial space with edema formation and a possible negative impact on postoperative organ function.

What We Already Know about This Topic

- The use of cardiopulmonary bypass during cardiac surgery may be associated with significant peri- and postoperative fluid loading and edema formation
- This study determined the transvascular fluid shift during cardiopulmonary bypass using isoflurane- ***versus*** propofol-based general anesthesia in a porcine model

What This Article Tells Us That Is New

- Isoflurane, in contrast to propofol, during cardiopulmonary bypass is associated with a greater increase in fluid extravasation from the intravascular to the interstitial space, resulting in dilution of interstitial fluid and a decrease in interstitial colloid osmotic pressure

THE use of cardiopulmonary bypass (CPB) during cardiac surgery is well known to be associated with significant postoperative fluid loading and edema formation[1] that occasionally contribute to myocardial, pulmonary, and splanchnic organ dysfunction. Restrictive perioperative fluid therapy during open heart surgery seems to be associated with improved postoperative organ function and recovery,[2] decreased incidence of postoperative complications,[3] and probably, shorter hospital stays in contrast to highly positive intraoperative fluid balance (>5 l) that has been associated with an adverse outcome.[3]

Factors contributing to fluid overloading during CPB and concomitant cardiac surgery are hemodilution,[4] the presence of a general inflammatory reaction related to contact between blood and foreign surfaces of the CPB circuit,[5] and the use of different levels of hypothermia[1,6] together with applied CPB-perfusion flow rate[7] and flow pattern (pulsatile/nonpulsatile).[8] Finally, the applied anesthetic technique may play a role in fluid homeostasis during anesthesia and surgery.

Numerous studies have been focused on changes in vascular permeability caused by volatile anesthetic agents.[9] Volume kinetic analysis after crystalloid fluid loading has identified an increase in interstitial fluid volume, during isoflurane

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anesthesia.[10] An isoflurane-related increase of the alveolar epithelial permeability and an increase of transendothelial albumin permeability have been observed after administration of isoflurane, but not with sevoflurane.[11,12] Similarly, administration of isoflurane (1–3%) has been shown to contribute to an opening of the blood–brain barrier.[13]

In a prospective observational study on incidence and risk factors of post-CPB hypoxemia by Weiss *et al.*[14] significantly lower Pa_{O_2}/F_{iO_2} ratios were observed 1 and 6 h following CPB only in patients with isoflurane anesthesia and not when an alternative anesthetic, enflurane, was administered. Recently, the authors commented that isoflurane-induced interstitial edema probably could have contributed to their previous observation.[15]

Volatile anesthetics are commonly used during CPB to maintain anesthesia and control arterial blood pressure. By lowering the systemic vascular resistance, they may interfere with the driving forces of the Starling equation controlling microvascular fluid- and protein-shifts between the vascular and the interstitial compartments. Theoretically, all these agents can increase the capillary hydrostatic pressure. Fluid retention seems, however, to be more associated with isoflurane than with enflurane, sevoflurane, or propofol.[11,15,16]

The current study was undertaken to describe in more detail the transvascular fluid shift during CPB with isoflurane- as compared with propofol-based general anesthesia in a porcine model. The animal model has previously been used in numerous studies on CPB and fluid homeostasis under normothermic, tepid, and hypothermic conditions.[1,4,6,7]

Materials and Methods

Animal Handling and Anesthesia

Fourteen immature domestic pigs, approximately 3 months old, of either sex, (Norwegian landrace Norhybrid; Stend Agricultural College, Bergen, Norway) were studied. The animals were acclimatized for at least 1 week in our laboratory housing area before the experiments. The anesthetic and experimental protocols were approved by the local laboratory animal veterinarian (Vivarium, University of Bergen, Bergen, Norway) under surveillance of the Norwegian Animal Research Authority (Oslo, Norway) and in accordance with national and international laws and regulations. Food was withdrawn 12 h before the study. Water was available at all times.

Anesthesia was carried out with preanesthetic medication of 500 mg of ketamine, 10 mg of diazepam, and 1 mg of atropine given as an intramuscular injection.[17] Thirty minutes later, general anesthesia was induced *via* a face mask with isoflurane in oxygen. After insertion of an ear vein catheter, anesthesia was supplemented by 5 mg/kg of thiopental intravenously before intubation of the trachea (blue line oral tube ID 6.0; Mallinckrodt, Covidien, Dublin, Ireland). All animals were thereafter ventilated (volume controlled) to an end-tidal carbon dioxide level of approximately 5.0 kPa. Anesthesia was maintained according to their study group allocation.

A midline sternotomy was performed and preparation for extracorporeal circulation was done. In parallel, arterial and venous lines for continuous monitoring of systemic arterial pressure and central venous pressure (CVP) were placed in the right femoral artery plus femoral vein and the right atrium (18-gauge; Secalone-TY™, BD Medical, Singapore, Singapore). A urinary bladder catheter was inserted *via* a midline mini-laparotomy. Surgery was normally completed within 20 min. All animals were thereafter allowed for 60-min stabilization before they underwent 120 min of tepid CPB.

Study Design and Groups

Fourteen animals were randomized into two study groups, the isoflurane-group (I-group; n = 7) and the propofol-group (P-group; n = 7). In the I-group, anesthesia was maintained by inhalation of 1.3% isoflurane in 50% oxygen in air. Adjustments in the inspired isoflurane concentration were allowed in the range of 0.5–2.0 vol%. During CPB, isoflurane 0.5% was added to the machine oxygenator. In the P-group, an infusion of propofol (Propolipid®; Fresenius Kabi, Stockholm, Sweden) was started at a level of 10–20 $mg \cdot kg^{-1} \cdot h^{-1}$. During the experiments, the dose was adjusted to 6 $mg \cdot kg^{-1} \cdot h^{-1}$ to maintain mean arterial blood pressure in the range of 50–80 mmHg, similar to that obtained with isoflurane. If necessary, repeated boluses of propofol were administered. Both study groups received a continuous intravenous infusion of fentanyl (7.5 $\mu g \cdot kg^{-1} \cdot h^{-1}$) and midazolam (0.5 $mg \cdot kg^{-1} \cdot h^{-1}$) according to a previous published protocol.[18] To avoid shivering during tepid CPB, all animals received an additional infusion of pancuronium of 0.45 $\mu g \cdot kg^{-1} \cdot h^{-1}$.

Acetated Ringer's solution (5 $ml \cdot kg^{-1} \cdot h^{-1}$) was administered as a maintenance fluid throughout the experiments in both study groups. Blood loss was substituted by the use of acetated Ringer's solution in volumes three times the recorded blood loss. During CPB, bleeding to the open chest was returned to circulation *via* the extracorporeal machine reservoir.

CPB

Cannulation for CPB was established in the ascending aorta and the right atrium with a 18-French aortic arch elongated one-piece arterial cannula (Medtronic, Minneapolis, MN) and a 32-French single-stage venous drainage cannula (TF-032-L; Research Medical Inc., Edwards Lifesciences Corp., Irvine, CA) connected to standard equipment for open heart surgery. A membrane oxygenator with reservoir and integrated heat exchanger (Quadrox, hollow fibre membrane oxygenator with heat exchanger and venous hardshell cardiotomy reservoir, VHK 4200; Jostra AG, Hirrlingen, Germany) was used. Pump flow was set to 2.7 $l \cdot m^{-2} \cdot min^{-1}$. Once the calculated CPB flow rate was

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achieved, ventricular fibrillation was induced with a 9 V DC battery set onto the right ventricle to obtain constant and controlled total body perfusion. CPB pump head pressures were in the range of 200–250 mmHg, and nonpulsatile flow was used. Inline arterial and venous measurement of oxygen tension was performed with a Po_2 monitor (Terumo CDI 500 in-line Blood Parameter Monitor System; Terumo Cardiovascular System Corp., Tustin, CA).

Before starting CPB, heparin 6 mg/kg body weight was administered intravenously, and after 1 h, an additional 3 mg/kg was given. Throughout the experiments, there was a constant fixed height difference (73 ± 3 cm) between the level of the machine reservoir and the site of venous drainage (the right atrium).

Body temperature was allowed to drift during CPB. Nasopharyngeal, rectal, and pulmonary artery temperatures were measured continuously.

The priming volume of the CPB circuit was 1,115 ml of acetated Ringer's solution which regularly resulted in a filling of the machine reservoir to a level of 400 ml. This level was used as a fluid gauge. Changes in this level indicated loss or gain of fluid from circulation to the interstitial space or changes in vascular tone. Whenever the level of the reservoir decreased, acetated Ringer's solution was added to restore the 400-ml level.

Hemodynamic Variables

Heart rate monitoring was obtained using surface electrocardiography electrodes. Mean arterial pressure and CVP from the femoral vein ($CVP_{fem.}$) and the right atrium ($CVP_{r.atr.}$) were recorded continuously using fluid-filled catheters connected to pressure transducers (Transpac II; Abbott Critical Care Systems, Sligo, Ireland) linked to a Hewlett Packard 78353A-monitor (Louisville, KY).

Blood Analyses and Plasma Colloid Osmotic Pressure

Blood samples were drawn from the arterial line for determination of hematocrit, plasma colloid osmotic pressure (COP_p), serum albumin, serum total protein and serum electrolyte concentrations, and acid–base parameters. Hematocrit was determined using standard hematocrit tubes centrifuged at 12,000 rpm for 10 min. Serum albumin and serum total protein concentrations were analyzed in an automatic analyzer (Technicon Chem. Systems; Technicon, Pittsburg, PA) by colorimetry and by the biuretic reaction, respectively.

COP_p was measured together with interstitial COP (COP_i) with a colloid osmometer using a semi-permeable membrane with a cutoff level at 10.000 Da (Amicon Inc., Beverly, MA) with acetated Ringer's solution in the reference chamber. The osmometer was designed to accept 5-μl sample volumes. Pressure was measured by a pressure transducer (Gould-Statham; Spectramed Inc., Levis Centre, Mount Vernon, OH) connected to a recorder (Easy-Graph 240; Gould Electronics Inc., Eastlake, OH).

Sampling of Interstitial Fluid

For determination of COP_i, fluid was sampled by means of multifilamentous nylon wicks (Number 18; Norsk Fletteri, Bergen, Norway). The wicks were soaked in acetated Ringer's solution before insertion. They were sewn into lateral abdominal skin folds by means of a straight suture needle (Acufirm 214/1, Dreieich, Germany) and placed subcutaneously at lengths of 8–10 cm. The protruding ends of the wicks were covered with an adhesive plastic film (Tegaderm, 3M Inc., London, Ontario, Canada) to prevent fluid loss by evaporation. The wicks were left *in situ* for 60 min, and after that they were pulled out swiftly and placed under mineral oil in centrifuge tubes.[18,19] Wick fluid (5–15 μl) was collected after centrifugation. Blood contamination in wicks was judged visually when removing each wick from the animal. Only white wicks were accepted.

Wick fluid from three implantation periods, before and twice during CPB, was analyzed.

Blood Volume Determination with the Carbon Monoxide Method

Erythrocytes volume and plasma volume (PV) were determined just before starting CPB by an indicator dilution technique using carbon monoxide as label.[20] Subsequent erythrocytes volume and PV were calculated every 30 min from recorded loss of blood and repeated determination of hematocrit. To assess the real *in vivo* PV of the animals during CPB, the calculated PV was corrected according to the following equation:

$$\text{PV in the animal (ml)} = \text{PV calculated (ml)} - \text{PV in the CPB circuit (ml)}$$

Fluid Loss and Supplementation, Fluid Extravasation

Urine output was recorded every half hour *via* a suprapubic catheter. All fluids administered to the animal were recorded including fluid additions to the machine reservoir during CPB. Net fluid balance (NFB), *i.e.*, all fluid additions minus diuresis and blood losses during CPB, was calculated for every interval of 30 min. NFB was used together with changes in PV (ΔPV) to calculate fluid extravasation (ml/kg) during a given time interval according to the formula:

$$\text{Fluidextravasation} = \text{NFB (ml/kg)} - \Delta\text{PV (ml/kg)}$$

Fluid extravasation rate (FER in $ml \cdot kg^{-1} \cdot min^{-1}$) was obtained by dividing fluid extravasation to the relevant time interval.

Total Tissue Water Content

At the end of each experiment, the pigs were sacrified by an intravenous injection of 20 ml of saturated KCl solution. Immediately thereafter, tissue samples (3 parallel pieces) were taken simultaneously from left quadriceps muscle, abdominal skin, colon, ileum, stomach, liver, pancreas, kidney, lung, heart, and brain. All samples were placed in preweighed vials,

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reweighed, and transferred to a drying chamber at 70°C. In the subsequent days, the vials were weighed repeatedly until stable weight. Total tissue water (TTW) was recorded as weight reduction in gram per gram dry weight. The values of the I-group and the P-group were compared with TTW values from a historic control group of 13 comparable pigs that never underwent CPB and were killed immediately after induction of pentobarbital anesthesia.

Statistics

Statistical analysis was performed with IBM SPSS Statistics version 20.0 for Windows (SPSS Inc., Chicago, IL). The results are presented as mean with SD in parentheses. A repeated measure ANOVA with one within-factor (time) and one between-factor (group) was used to analyze each of the variables in table 1 (hemodynamic parameters and PV), table 2 (laboratory parameters), and in the figures (fig. 1: hematocrit, COP_p, and interstitial fluid; fig. 2: NFB and FER). If the within-factor *P* value (P_{Time}) was less than 0.05, a multiple comparison procedure, according to Bonferroni's method, was conducted. Only the *P* value ($P_{post\ hoc}$) based on the comparison between the mean value at pre-CPB and at 120 min is presented in tables 1 and 2 and in figure 1. In figure 2, the comparison between the mean value at pre-CPB and during CPB was performed. Furthermore, a two-sample *t* test (p_b) was performed to compare the two groups at different time points (60 and 120 min). This was only done if the interaction *P* value ($P_{Group \times Time}$) was less than 0.05.

For the variables given in table 3, the one-way ANOVA was used to test the differences between three independent groups, followed by a Tukey *post hoc* test if a significant between-group *P* value was obtained. The chosen level of significance was set to 0.05.

Results

The animals of the study groups were comparable with respect to age, mean (SD): 89.9 (19.8) *versus* 93.0 (10.8) days, weight: 32.6 (2.2) *versus* 32.1 (2.3) kg, and sex: male/female: 5/2 *versus* 5/2, I-group *versus* P-group, respectively. The body core temperature was allowed to drift in both study groups from a normothermic to a tepid level during the experiments. The temperature at baseline (pulmonary artery) was 38.3° (0.6) and 38.5°C (0.6); at 30 min CPB, 36.7° (0.6) and 36.4°C (0.5); and at 120 min CPB, 36.3° (0.5) and 36.3°C (0.5), I-group *versus* P-group, respectively.

Hemodynamics, Plasma Volume, Colloid Osmotic Pressures

Mean arterial pressure, $CVP_{fem.}$, $CVP_{r.atr.}$, systemic vascular resistance, and PV before and during CPB are displayed in table 1. The animals remained hemodynamically stable for all parameters during the experiments with no between-group differences (table 1).

Similarly, no significant between-group differences were obtained in the investigated laboratory parameters (table 2) including hematocrit and COP_p (fig. 1, A and B). In both groups, a slight increase was observed in the s-chloride concentration during the experiments, whereas s-albumin and s-protein concentrations (table 2) together with hematocrit and COP_p (fig. 1, A and B) decreased after initiation of CPB related to hemodilution.

COP_i decreased significantly in the I-group after starting CPB, whereas COP_i remained stable throughout the experiments in the P-group. After 60 min of CPB, COP_i was significantly lower in the I-group ($P = 0.009$) as compared with the P-group (fig. 1C).

Table 1. Hemodynamic Parameters and Plasma Volume before and during CPB

Parameters	Group	Pre-CPB	60-min CPB	120-min CPB	P_{Time}	P_{Group}	$P_{Group \times Time}$	$P_{Post\ Hoc}$
MAP, mmHg	I	64.5 (19.8)	57.2 (11.5)	56.5 (9.8)	0.435	0.191	0.977	
	P	72.8 (33.5)	62.5 (8.9)	61.8 (9.9)				
$CVP_{fem.}$, mmHg	I	7.9 (3.7)	7.9 (4.1)	8.6 (3.9)	0.847	0.618	0.561	
	P	8.0 (3.2)	7.0 (2.8)	6.8 (1.7)				
$CVP_{r.atr.}$, mmHg	I	5.2 (2.1)	4.8 (2.1)	4.3 (2.1)	0.107	0.448	0.673	
	P	5.2 (3.1)	3.2 (3.3)	2.7 (3.2)				
SVR, dyn·s cm^{-5}	I	1,447 (322)	1,296 (298)	1,260 (268)	0.029	0.199	0.456	0.160
	P*	1,727 (213)	1,316 (322)	1,446 (260)				
PV, ml/kg	I	54.5 (3.5)	48.6 (8.0)	52.7 (7.1)	0.084	0.756	0.852	
	P	56.0 (5.6)	51.3 (13.6)	52.8 (14.1)				

Values are presented as mean with SD in parentheses.

* n = 5.

CPB = cardiopulmonary bypass; $CVP_{fem.}$ = central venous pressure measured in the right femoral vein; $CVP_{r.atr.}$ = central venous pressure measured in the right atrium; I = isoflurane group; MAP = mean arterial pressure, measured in the right femoral artery; P = propofol group; PV = plasma volume; SVR = systemic vascular resistance; P_{Group} = between-factor; $P_{Group \times Time}$ = interaction; $P_{posthoc}$ = *P* value comparing Pre-CPB and CPB at 120 min; P_{Time} = within-factor.

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Table 2. Laboratory Parameters before and during CPB

Parameters	Group	Pre-CPB	60-min CPB	120-min CPB	P_{Time}	P_{Group}	$P_{Group \times Time}$	$P_{Post\ Hoc}$
s-osmolality, mosm/l	I	287 (3.7)	286 (4.6)	287 (5.5)	0.366	0.845	0.816	
	P	286 (4.2)	286 (2.5)	287 (2.7)				
s-natrium, mM	I	137.7 (1.5)	138.5 (0.8)	137.8 (0.9)	0.067	0.022	0.630	
	P	138.7 (1.1)	139.7 (1.4)	139.6 (1.1)				
s-kalium, mM	I*	4.1 (0.3)	3.8 (0.4)	4.0 (0.4)	0.029	0.774	0.910	>0.9
	P	4.1 (0.2)	3.8 (0.4)	4.0 (0.3)				
s-chloride, mM	I*	96.8 (1.7)	101.3 (2.3)	101.5 (2.2)	<0.0001	0.180	0.189	<0.001
	P	99.7 (1.8)	102.4 (2.2)	102.1 (2.5)				
s-albumin, g/l	I	29.0 (3.2)	21.9 (4.8)	18.9 (3.5)	<0.0001	0.087	0.195	<0.001
	P	31.7 (2.7)	24.4 (4.4)	24.0 (4.2)				
s-protein, g/l	I	45.1 (3.8)	31.6 (4.9)	29.1 (3.5)	<0.0001	0.069	0.058	<0.001
	P	46.9 (3.3)	36.1 (5.2)	35.6 (5.2)				
Albumin mass, g	I	66.3 (21.1)	65.0 (17.2)	60.3 (15.4)	0.224	0.773	0.657	
	P	62.3 (11.1)	62.6 (5.1)	60.6 (3.9)				
Protein mass, g	I	80.3 (6.6)	77.1 (7.8)	77.1 (8.0)	0.080	0.030	0.483	
	P	90.6 (11.2)	89.5 (11.4)	89.6 (9.1)				
pH	I	7.51 (0.02)	7.44 (0.01)	7.46 (0.03)				
	P	7.49 (0.05)	7.44 (0.04)	7.45 (0.04)				
pCO2, kPa	I	5.0 (0.6)	5.8 (0.4)	5.8 (0.6)				
	P	5.1 (0.6)	5.7 (0.5)	6.0 (0.4)				
Base excess, mM	I	6.2 (1.4)	5.6 (1.1)	7.3 (0.8)				
	P	4.7 (1.6)	5.4 (2.6)	6.5 (3.1)				

All values are presented as mean with SD in parentheses at time 0 (Pre-CPB control values) and 60 and 120 min after initiation of CPB.
* n = 6.

CPB = cardiopulmonary bypass; I = isoflurane group; P = propofol group; P_{Group} = between-factor; $P_{Group \times Time}$ = interaction; $P_{post\ hoc}$ = *P* value comparing Pre-CPB and CPB at 120 min; P_{Time} = within-factor.

Net Fluid Balance, Fluid Extravasation, and TTW content

NFB in the P-group showed a modest increase from 0.16 (0.09) $ml \cdot kg^{-1} \cdot min^{-1}$ before CPB to 0.40 (0.29) $ml \cdot kg^{-1} \cdot min^{-1}$ during CPB. NFB in the I-group increased from a similar value of 0.11 (0.02) $ml \cdot kg^{-1} \cdot min^{-1}$ before CPB to 0.84 (0.30) $ml \cdot kg^{-1} \cdot min^{-1}$ during CPB. During CPB, significant between-group differences in NFB were observed with the higher values in the I-group (p_b = 0.014; fig. 2A).

Fluid losses from circulation to the interstitial space presented as FER in $ml \cdot kg^{-1} \cdot min^{-1}$ are presented in figure 2B. FER in the I-group and P-group before CPB was 0.27 (0.13) and 0.28 (0.17) $ml \cdot kg^{-1} \cdot min^{-1}$, respectively. During CPB, FER remained unchanged at 0.32 (0.19) $ml \cdot kg^{-1} \cdot min^{-1}$ in the P-group, whereas an increase to a level of 0.92 (0.36) $ml \cdot kg^{-1} \cdot min^{-1}$ was observed in the I-group. The FER values of the I-group were significantly higher than the values of the P-group during CPB (p_b = 0.002; fig. 2B).

TTW of the different organs studied is presented in table 3. For comparison, all values were compared with historic control data obtained from our laboratory comparable pigs that never underwent CPB and were sacrified immediately after initiation of anesthesia. TTW of the P-group was increased in some of the gastrointestinal organs and the heart when compared with the control animals. The I-group showed an increase in TTW in most organs when compared with the control animals. Comparison of the two study groups revealed higher water content in the kidneys, stomach, intestine, skeletal muscle, and skin of the I-group (table 3).

Discussion

In the current study, isoflurane was compared with propofol in an established experimental model for studies on fluid homeostasis and fluid shifts during CPB. This study confirms the hypothesis that general anesthesia with isoflurane, in contrast to propofol, contributes to a significant, in fact three-fold, increase in the FER affecting most tissues and organs during CPB. Hematocrit, COP_p, and s-protein concentrations were similar in the two study groups indicating that different degrees of hemodilution could not explain the results. Furthermore, no differences in temperature were present excluding hypothermia as an explanation for the fluid accumulation seen in the I-group.

The increase in FER is reflected by significant dilution of the interstitial fluid after initiation of CPB with a decrease in COP_i in the animals given isoflurane, whereas COP_i remained unchanged and stable in the propofol-treated animals.

Similarly, changes in TTW in the I-group are in line with a significant increase in fluid extravasation when compared with the P-group. The clinically significant increase in

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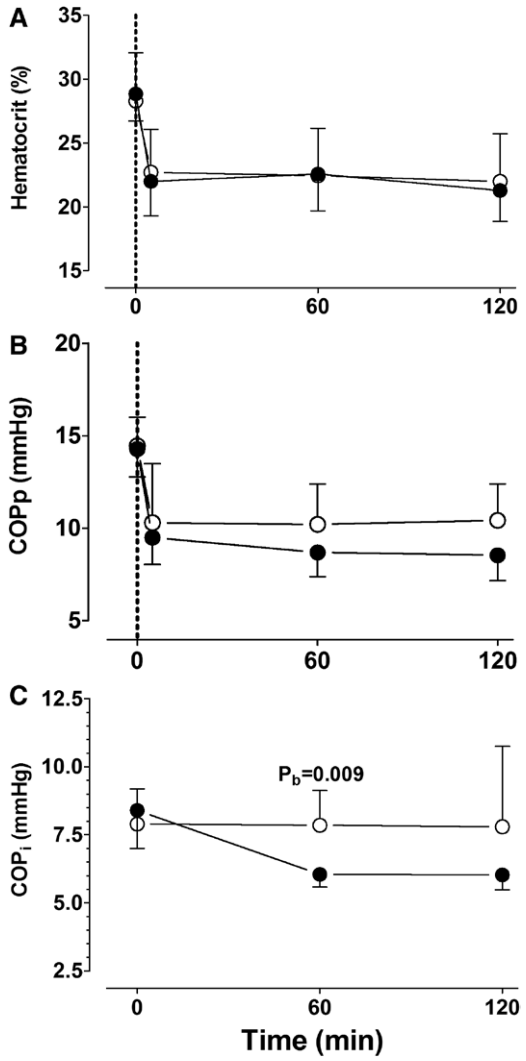

Fig. 1. (*A*) Hematocrit in percent, before and during 120 min of cardiopulmonary bypass. *Closed circles*: isoflurane-group (I-group); *open circles*: propofol-group (P-group). The data represent the mean values with SD from seven animals in each group. A repeated measure ANOVA with one within-factor (time) and one between-factor (group) was used to analyze each of the variables. If the within-factor *P* value (P_{Time}) was less than 0.05, a multiple comparison procedure according to Bonferroni method was conducted. $P_{Time} < 0.0001$; P_{Group}: 0.909; $P_{Time \times Group}$: 0.134; $P_{post\ hoc} < 0.0001$. (*B*) Colloid osmotic pressure in plasma (COP_p) in mmHg, before and during 120 min of cardiopulmonary bypass. *Closed circles*: isoflurane-group (I-group); *open circles*: propofol-group (P-group). The data represent the mean values with SD from seven animals in each group. A repeated measure ANOVA with one within-factor (time) and one between-factor (group) was used to analyze each of the variables. If the within-factor *P* value (P_{Time}) was less than 0.05, a multiple comparison procedure, according to Bonferroni method, was conducted. $P_{Time} < 0.0001$; P_{Group}: 0.267; $P_{Time \times Group}$: 0.026; $P_{Post\ hoc} < 0.0001$. A two-sample *t* test was performed to compare the two groups at time point 120 min. Between-group difference (p_b) at time 120 min is reported ($p_b = 0.06$). (*C*) Colloid osmotic pressure in interstitial fluid (COP_i) in mmHg, before, and during 120 min of cardiopulmonary bypass. *Closed circles*: isoflurane-group (I-group); *open circles*: propofol-group (P-group). The data represent the mean values with SD from six animals in each group. A repeated measure ANOVA with one within-factor (time) and one between-factor (group) was used to analyze each of the variables. If the within-factor *P* value (P_{Time}) was less than 0.05, a multiple comparison procedure, according to Bonferroni method, was conducted. $P_{Time} < 0.029$; P_{Group}: 0.178; $P_{Time \times Group}$: 0.041; $P_{post\ hoc} < 0.209$. A two-sample *t* test was performed to compare the two groups at time point 60 min. Between-group difference (p_b) at time 60 min is reported ($p_b = 0.009$).

TTW in skeletal muscle and skin is interesting because the TTW increase in these major organ systems is pointing on the magnitude of fluid shifts during 120 min of CPB in the I-group. Both study groups had higher tissue water content than the historic controls, probably due to various factors such as anesthesia, surgery, and CPB.

Lung function has been demonstrated to be negatively affected by volatile anesthetics. Destabilization of

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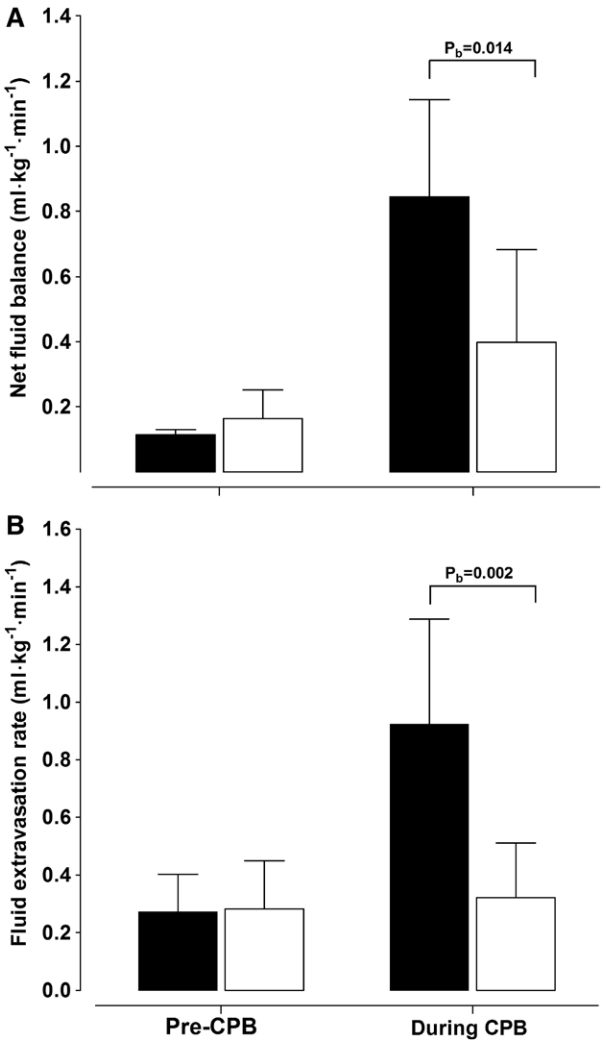

Fig. 2. (*A*) Net fluid balance before and during (*i.e.*, 0–120 min) cardiopulmonary bypass (CPB) presented as ml·kg^{-1}·min^{-1}. *Black columns*: isoflurane-group (I-group); *white columns*: propofol-group (P-group). The data represent the mean values with SD from seven animals in each group. A repeated measure ANOVA with one within-factor (time) and one between-factor (group) was used to analyze each of the variables: P_{Time} < 0.0001; P_{Group}: 0.036; $P_{Time \times Group}$: 0.006. A two-sample *t* test was performed to compare the two groups during CPB. Between-group difference (p_b) at time 120 min is reported (p_b = 0.014). (*B*) Fluid extravasation rate before and during (*i.e.*, 0–120 min) CPB presented as ml·kg^{-1}·min^{-1}. *Black columns*: isoflurane-group (I-group); *white columns*: propofol-group (P-group). The data represent the mean values with SD from seven animals in each group. A repeated measure ANOVA with one within-factor (time) and one between-factor (group) was used to analyze each of the variables: P_{Time} < 0.0001; P_{Group}: 0.013; $P_{Time \times Group}$: 0.001. A two-sample *t* test was performed to compare the two groups during CPB. Between-group difference (p_b) at time 120 min is reported (p_b = 0.002).

surfactant,[21] alveolar epithelial injury,[12] and effects on fluid homeostasis[14,15] may play a role. Isoflurane has been found to increase the permeability of the alveolocapillary barrier.[22] The mechanisms behind this phenomenon remains unclear, but could be related to isoflurane-initiated release of vascular endothelial growth factor or to synthesized nitric oxide contributing to vasodilatation and an increase in vascular permeability.[23]

As demonstrated in this study, an increase in vascular permeability seems not to be limited to the pulmonary vasculature alone, but to affect capillaries in other organs of the body as well. With sevoflurane, such effects have been claimed to be absent.[22]

In the current study, a trend to higher TTW of the lungs were observed in the I-group as compared with the P-group with a numeric difference of 25%. The lack of significant differences may be explained by ongoing contractions of the right ventricle during bypass, leading to various degrees of perfusion/hypoperfusion in the pulmonary vessels during CPB resulting in lowered and variable capillary hydrostatic pressure and corresponding variation in basic FER.

Vascular fluid shifts are well described by the Starling equation focusing on the physiological mechanisms involved in fluid transfer between the vascular and the extravascular compartments: $Jv = CFC \cdot (P_c - P_i) - \sigma \cdot (COP_p - COP_i)$.[24] Fluid filtration (Jv) depends on the capillary filtration coefficient (CFC) which is proportional to hydraulic conductivity and capillary area accessible for fluid transfer. P_c and P_i describe the capillary hydrostatic pressure (c) and the interstitial fluid hydrostatic pressure (i). COP_p and COP_i describe the COPs of the respective compartments. The capillary hydrostatic pressure (P_c) decreases in humans from a level of approximately 32–36 mmHg at the arterial end to levels of 12–25 mmHg at the venous end of the capillaries, and the P_c is generally lower in the lungs and in hepatic sinusoids.[24]

Similar conditions are assumed to exist in pigs. P_c is partly influenced by the sympathetic tone reflected by systemic vascular resistance. In the current study, systemic vascular resistance of the I-group tended to be below the values of the P-group before and partly during CPB, suggesting lower degree of precapillary resistance and higher P_c values in the I-group, which may have contributed to an increase in FER.

P_c is, however, also strongly influenced by the venous pressure (P_v) according to the equation: $P_c = P_v + F \bullet R_v$ where F is blood flow and R_v is venous resistance.[25] In fact P_c is four times more sensitive to the venous pressure than to the arterial pressure.[24] In the current study, there was a slight nonsignificant trend to higher CVP values in the I-group that additionally may have contributed to an increase in P_c with an increase in FER.

The presence of an isoflurane-related increase in TTW content in capsulated organs such as liver and kidneys is peculiar. In the liver, nearly three quarters of the hepatic blood flow occurs at near venous pressure with values approximately 6 mmHg in the hepatic sinuoides.[24] The trend to higher CVP values of the I-group compared with the P-group may have affected both hepatic blood flow and

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Table 3. Total Tissue Water Content after 120 min of CPB

Tissue	C-Group (n = 13)	P-Group (n = 7)	I-Group (n = 7)	P_{Group}	$P_{Post\ Hoc}$(P *vs.* C)	$P_{Post\ Hoc}$(I *vs.* C)	$P_{Post\ Hoc}$(P *vs.* I)
Right myocardium	4.17 (0.09)	4.54 (0.31)	4.71 (0.40)	<0.0001	0.013	<0.0001	0.454
Left myocardium	4.00 (0.16)	4.31 (0.34)	4.33 (0.16)	0.003	0.015	0.009	0.982
Lung	4.24 (0.38)	6.36 (1.19)	7.97 (2.38)	<0.0001	0.008	<0.0001	0.087
Liver	2.89 (0.19)	2.92 (0.15)	3.19 (0.13)	0.002	0.885	0.002	0.017
Left kidney	4.45 (0.26)	4.26 (0.26)	5.20 (1.02)	0.008	0.748	0.023	0.012
Right kidney	4.56 (0.30)	4.25 (0.21)	4.71 (0.37)	0.023	0.081	0.569	0.022
Stomach (muscularis)	4.03 (0.33)	4.94 (0.53)	5.80 (0.79)	0.001	0.003	<0.0001	0.015
Stomach (mucosa)	4.34 (0.25)	4.32 (0.33)	5.20 (0.73)	<0.0001	0.997	0.001	0.003
Pancreas	3.31 (0.45)	3.90 (0.38)	4.14 (0.46)	<0.0001	0.021	0.001	0.525
Ileum (muscularis)	3.77 (0.57)	4.46 (0.37)	4.63 (0.48)	<0.0001	0.020	0.003	0.790
Ileum (mucosa)	4.59 (0.51)	4.68 (0.26)	5.68 (0.39)	0.015	0.906	<0.0001	0.001
Colon	3.85 (0.68)	4.62 (1.29)	5.41 (0.92)	0.005	0.200	0.004	0.263
Skeletal muscle	3.43 (0.13)	3.73 (0.12)	3.89 (0.17)	<0.0001	<0.0001	<0.0001	0.094
Skin	1.93 (0.26)	2.28 (0.42)	2.83 (0.57)	<0.0001	0.166	<0.0001	0.044
Brain	3.74 (0.25)*	3.87 (0.26)	3.81 (0.21)	0.597			

Total tissue water content (gram per gram of dry weight) in the different tissues at the end of hypothermic extracorporeal circulation. Values are given as mean with SD in parentheses.

* n = 7.

CPB = cardiopulmonary bypass; C-group = control animals that never underwent CPB; I-group = isoflurane-based anesthesia; P-group = propofol-based anesthesia.

the capillary hydrostatic filtration pressure and may thereby have contributed to the increased TTW levels of the liver during isoflurane anesthesia. Similar conditions could also be present in the kidneys and thereby have contributed to an increase in TTW in these organs as well.

The clinical relevance of these results are emphasized by reports in the literature of an association between perioperative fluid overloading and postoperative adverse outcome seen in cardiac,[2,3] abdominal,[26,27] and pulmonary surgery.[28] Significant perioperative fluid accumulation with formation of tissue edema may contribute to postoperative organ dysfunction, whereas fluid restriction seems to be associated with better organ performance as recently demonstrated by Kvalheim *et al.*[29] concerning heart and lung function.

The blood–brain barrier may also be affected by anesthesia with isoflurane. Tétrault *et al.*[13] reported breakdown of the blood–brain barrier in cortex and thalamus after 3% isoflurane anesthesia, whereas 1% isoflurane only resulted in an opening of the blood–brain barrier in thalamus with an increase in cerebral volume. The current experiments were performed with isoflurane concentrations commonly at a level of 0.5–1.5% given before and during CPB. The study was not designed to evaluate the patency of the blood–brain barrier in the research animals, and no differences in TTW of the brains were seen. However, brain tissue was only sampled from the cortex. In future studies, the water content of the central brain, such as the thalamus, should be included.

Limitations of the Study

Our study may have several important limitations. First of all, the number of animals included in each study group is small. FER and COP_i are the main variables in the current study. From a number of previous experimental studies with similar size, *post hoc* power analyses with focus on FER and COP_i have demonstrated a power of the results in the order of 90–95%.[1,30,31] Based on these results and partly related ethical considerations concerning the use of animals in experimental laboratory research, the number of seven animals per study group was chosen. A *post hoc* power analysis of the results of the above-mentioned parameters in the current study confirmed a power more than 90% for FER and COP_i.

According to the study protocol, anesthesia was induced in both study groups by administration of isoflurane before venous access was established. Theoretically, the results could have been influenced by the fact that isoflurane may cause a prolonged increase in alveolar epithelial permeability.[32] However, this bias would only contribute to diminish the difference between the groups with respect to FER and TTW.

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The intravenous anesthetic agent, propofol, is well known to produce marked vasodilatation *in vivo* related to its nitric oxide–releasing properties. In a previous study on systemic hemodynamic response, similar changes were seen after induction of anesthesia with isoflurane and propofol, intubation, skin incision, and sternotomy.[33] The exception was a more pronounced decrease in systemic vascular resistances after propofol administration. The current study revealed a different pattern with trend to lower mean arterial pressure levels in the I-group throughout the experiment. This may represent a limitation to the current study because it could be due to differences in hemodynamic response between human and pigs or to the use of nonequivalent doses of propofol between these studies.

To conclude: isoflurane in contrast to propofol anesthesia during CPB is associated with an increase in the extravasation of fluid from the intravascular to the interstitial space resulting in dilution of interstitial fluid and a decrease in interstitial COP. The resulting formation of tissue/organ edema may negatively affect vital organ functions. Implications of these findings have to be more extensively evaluated in experimental models and in a clinical setting.

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