Acute Normovolemic Hemodilution in the Pig Is Associated with Renal Tissue Edema, Impaired Renal Microvascular Oxygenation, and Functional Loss

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ABSTRACT

Background: The authors investigated the impact of acute normovolemic hemodilution (ANH) on intrarenal oxygenation and its functional short-term consequences in pigs.

Methods: Renal microvascular oxygenation (μP_{O_2}) was measured in cortex, outer and inner medulla *via* three implanted optical fibers by oxygen-dependent quenching of phosphorescence. Besides systemic hemodynamics, renal function, histopathology, and hypoxia-inducible factor-1α expression were determined. ANH was performed in n = 18 pigs with either colloids (hydroxyethyl starch 6% 130/0.4) or crystalloids (full electrolyte solution), in three

What We Already Know about This Topic

- As more restrictive transfusion is advanced, hemodilution and its influence on acute kidney injury are of increasing importance
- Intrarenal hypoxia is likely one factor contributing to acute kidney injury during the perioperative period

What This Article Tells Us That Is New

- Compared with colloid (hydroxyethyl starch 6% 130/0.4), normovolemic hemodilution with crystalloid (balanced electrolyte solution) produced greater renal hypoxia indicating the diluent may be a factor when evaluating hemodilution and acute kidney injury

steps from a hematocrit of 30% at baseline to a hematocrit of 15% (H3).

Results: ANH with crystalloids decreased μP_{O_2} in cortex and outer medulla approximately by 65% ($P < 0.05$) and in inner medulla by 30% ($P < 0.05$) from baseline to H3. In contrast, μP_{O_2} remained unaltered during ANH with colloids. Furthermore, renal function decreased by approximately 45% from baseline to H3 ($P < 0.05$) only in the crystalloid group. Three times more volume of crystalloids was administered compared with the colloid group. Alterations in systemic and renal regional hemodynamics, oxygen delivery and oxygen consumption during ANH, gave no obvious explanation for the deterioration of μP_{O_2} in the crystalloid group. However, ANH with crystalloids was associated with the highest formation of renal tissue edema and the highest expression of hypoxia-inducible factor-1α, which was mainly localized in distal convoluted tubules.

Conclusions: ANH to a hematocrit of 15% statistically significantly impaired μP_{O_2} and renal function in the crystalloid

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group. Less tissue edema formation and an unimpaired renal μP_{O_2} in the colloid group might account for a preserved renal function.

NORMOVOLEMIC hemodilution with crystalloid or colloid solutions is a common technique to reduce the requirements for allogeneic blood transfusion. Hemodilution can be the result of fluid resuscitation in emergency situations[1] and of volume therapy in the daily routine.[2,3] There are not much data on the influence of hemodilution on renal function. In previous investigations, hemodilution has been identified as an important risk factor in the development of acute kidney injury (AKI) for patients undergoing cardiopulmonary bypass.[4,5] However, transposition of data observed in cardiac surgery is quite speculative as clinical conditions may vary in other surgical contexts. Therefore, more research into the relationship between hemodilution and the occurrence of AKI is necessary.

One reason for the development of AKI after hemodilution could be a high renal sensitivity to decreased hematocrit. Although under physiological circumstances the kidney's oxygen delivery (DO_2) exceeds its oxygen consumption by far,[6] our group could demonstrate an immediate impairment in renal cortical and outer medullary microvascular oxygenation and a supply dependency for oxygen, when hematocrit started to drop during acute normovolemic hemodilution (ANH) in the rat.[7] There was a correlation between renal oxygen consumption (VO_2) and microvascular P_{O_2} (μP_{O_2}) during reduction in hematocrit, which was accompanied by an increase in intrarenal oxygen shunt. Therefore, our previous results demonstrated that the renal oxygen supply of the kidney became critical already in an early stage of ANH.

There is more and more evidence that a disturbed intrarenal oxygenation plays an important role in the development of AKI,[8] although a direct link between renal hypoxia and functional loss could not yet been identified. Recent studies indicate that renal hypoxia can stimulate fibrogenesis with the consequence of glomerular and tubulointestitial scarring.[9] The activation of hypoxia-inducible factor (HIF) is an oxygen-dependent mechanism to prevent renal damage.[10] However, increased HIF expression, caused by low partial pressure of oxygen, may itself induce fibrosis and glomerular damage.[11]

The current study was designed to investigate the impact of ANH on intrarenal oxygenation and its short-term functional consequences. We performed our experiments on a porcine model of ANH, because these animals have been demonstrated to have similar physiological responses compared with humans.[12] We hypothesized that ANH impairs renal oxygenation and kidney function in the pig. ANH was performed to a hematocrit of 15% and the use of colloid *versus* crystalloid solutions was compared.

Materials and Methods

Animals

The protocol of the current study was approved by the Animal Research Committee of the Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands (DEC Nr. EMC 2041 [EMC Nr. 129-10-01]). Animal care and handling were performed in accordance with the national guidelines for care of laboratory animals. For the experiments 18 female crossbred Landrace × Yorkshire pigs, with mean body weights of 30.5 ± 1 kg (mean ± standard error) were used.

Experimental Preparation

After an overnight fast with free access to water, the animals were sedated with an intramuscular injection of ketamine (30 mg/kg; Alfasan, Woerden, The Netherlands) and midazolam (1 mg/kg; Roche, Mijdrecht, The Netherlands). Tracheotomy was performed under continuous infusion of ketamine (17 mg·kg^{-1}·h^{-1}) and midazolam (1.25 mg·kg^{-1}·h^{-1}). After tracheotomy, sufentanil (8 µg·kg^{-1}·h^{-1}; Janssen-Cilag, Tilburg, The Netherlands) was administered as an analgesic agent. Muscle relaxation was obtained by single-dose of pancuronium bromide (0.3 mg/kg; Organon, Oss, The Netherlands) before laparotomy.

Mechanical ventilation (Servo 300; Siemens-Elema, Solna, Sweden) was performed standardized with an $F_{I_{O_2}}$ of 30% and maintaining normocapnia (arterial carbon dioxide tension, 40 mmHg). Central body temperature was maintained at approximately 38°C with two heating pads under and a heating lamp above the animal. A crystalloid solution (Sterofundin; B. Braun, Melsungen, Germany) was administered (15 ml·kg^{-1}·h^{-1}) as maintenance fluid.

Two peripheral catheters were placed in the ears for administration of fluids and anesthesia. One line (20G; Vygon, Écouen, France) was inserted in the left internal carotid artery for measuring arterial blood pressure and for collection of blood samples. A pulmonary artery thermodilution catheter (Edwards 7-French; Baxter Healthcare Corp., Round Lake, IL) was positioned in the pulmonary artery *via* an introducer in the right jugular vein allowing the measurement of central venous pressure (CVP), pulmonary artery, and pulmonary capillary wedge pressure, central body temperature, continuous cardiac output, and collection of central venous and mixed venous blood samples.

After midline laparotomy, the right kidney was mobilized and fixed in a custom-made bucket. The renal vessels were carefully separated from each other to ensure preservation of the nerves. A flow-probe (4SB; Transonic Systems Inc., Ihtaca, NY) around the renal artery allowed the continuous measurement of renal blood flow (RBF) and a catheter in the renal vein enabled the collection of renal venous blood samples for blood gas analysis. The ureter of the right kidney was isolated, ligated, and cannulated with a polyethylene catheter for urine samples. For measurement of intrarenal microvascular oxygenation an optical fiber was inserted in the cortex, in the outer and in the inner medulla, and a

detection fiber was placed 1 mm above kidney surface. The bladder urine, deriving from the left kidney, was collected *via* an implanted catheter. To prevent evaporation of body fluids *via* the surgical field, the abdomen was closed with surgical clips. During the experiment the animal's response to pain stimulus by clamping the paw on regular time points was tested. None of the animals showed muscular response, increase in heart rate or blood pressure. At the end of the experiment, the kidney was removed, weighed, and the position of all inserted probes were controlled for correct placement. Furthermore, samples of renal tissue were fixed in formaldehyde for histological analysis. Animals were sacrificed by an infusion of potassium chloride 7.4%, 40 ml, which caused sudden cardiac arrest.

Hemodynamic and Blood (Gas) Measurements

Mean arterial pressure (MAP), heart rate, CVP (Siemens Monitor SC 9000; Siemens AG, Erlangen, Germany), cardiac output (Vigilance Monitoring; Edwards Lifesciences, Irvine, CA) and RBF (T206; Transonic Systems Inc.) were measured and recorded continuously. Blood samples (0.5 ml) were taken at four different time points: after operation and stabilization time = baseline, after the first step of hemodilution = H1, after the second hemodilution step = H2, and after the last step of hemodilution = H3. Arterial, central venous, mixed venous, and renal venous samples were taken and analyzed for blood gas values, hematocrit and, serum lactate (ABL Flex 800, Radiometer, Copenhagen, Denmark). Samples were also used to determine sodium, potassium, plasma creatinine, and plasma inulin concentrations.

Intrarenal Microvascular Oxygen Measurements

The technique of oxygen-dependent quenching of phosphorescence was applied for measurement of the intrarenal microvascular oxygenation. The animal received an infusion of Pd-porphyrin (Pd-*meso*-tetra(4-carboxyphenyl)porphine; Porphyrin Products, Logan, UT) bound to albumin.[13,14] When Pd-porphyrin is excited by laser light, the phosphorescence intensity decreases at a rate dependent on the surrounding oxygen concentration.[15] The relationship between the measured decay time and the partial pressure of oxygen (Po_2) is given as: $Po_2 = (1/\tau - 1/\tau_0)/k_q$, where τ is the measured decay time, τ_0 is the decay time at an oxygen concentration of zero, and k_q is the quenching constant.

The excitation source was an Opolette 355-I (Opotek, Carlsbad, CA), a compact computer–controlled tunable laser providing pulses with a specified duration of 4–10 ns and typically 2–4 mJ/pulse over the tunable range of 410–670 nm. Excitation of the dye in renal cortex, outer and inner renal medulla was performed *via* three optical fibers (500 µm diameter, MT B500-0 L240; Perimed, Järfälla, Sweden) inserted in the corresponding depth of the kidney. The detection fiber was a liquid light guide (Model 77639; Newport, Irvine, CA). The detector was a photomultiplier tube with a combination of a 630 nm longpass filter

(OG590; Newport) and a 690 nm bandpass filter (Omega Optical, Brattleboro, VT). Data-acquisition software was written in LabView (Version 7.1; National Instruments, Austin, TX). A detailed description of the used laser and the setup can be found elsewhere.[16]

Calculation of Renal Oxygen Delivery, Oxygen Consumption, Oxygen Extraction Ratio, and Vascular Resistance

Renal DO$_2$ (DO$_{2ren}$ in ml/min) was calculated as DO$_{2ren}$ = RBF × (1.31 × Hb × S$_a$O$_2$) + (0.003 × Pao$_2$), where (1.31 × Hb × S$_a$O$_2$) + (0.003 × Pao$_2$) is the arterial oxygen content. Renal VO$_2$ (VO$_{2ren}$ in ml/min) was calculated as follows VO$_{2ren}$ = RBF × (arterial – venous oxygen content), where renal venous oxygen content was calculated as (1.31 × Hb × S$_{rv}$O$_2$) + (0.003 × P$_{rv}$O$_2$). The renal oxygen extraction ratio (O$_2$ER$_{ren}$ in %) was calculated as O$_2$ER$_{ren}$ = VO$_{2ren}$/ DO$_{2ren}$. The renal vascular resistance was calculated as: (MAP – CVP)/RBF.

Kidney Function

Creatinine and inulin clearance were determinates as an index of glomerular filtration rate. Both clearances were calculated using the standard formula clearance (ml/min) = (*U* × *V*)/*P*. *U* stands for urine concentration (either creatinine or inulin), *V* for volume per time, and *P* for plasma concentration (either creatinine or inulin). The clearances were only calculated for the right kidney. Urine samples were collected from the right kidney at intervals of 20 min for analysis of urine volume and urine concentration. At the midpoint of each urine-sampling period plasma samples were collected. The concentrations of creatinine and inulin in urine and plasma were determined by colorimetric methods.

Urinary Neutrophil Gelatinase-associated Lipocalin concentration

Urinary neutrophil gelatinase-associated lipocalin (NGAL) was determined with an enzyme-linked immunosorbent assay kit (KIT 044; BioPorto Diagnostics, Gentofte, Denmark).[17] Urine samples were stored at –80°C until analysis. Briefly, a precoated 96-microwell plate with a monoclonal antibody against Pig-NGAL was used. For determination of the NGAL concentration a calibration curve was constructed using calibrator solution (range 0–400 pg/ml). Diluted urine samples (1:1,000) were added to the precoated wells incubated for 1 h at room temperature and washed. In the next four steps biotinylated Pig-NGAL antibody, horseradish peroxidase–streptavidin, tetramethyl benzidine-substrate, and stop solution were add to the microwells for incubation and washed when applicable. The quantity of color intensity was read at 450 nm using an ELISA reader (VersaMax; Molecular Devices, Sunnyvale, CA). Urinary NGAL concentration was calculated from the calibration curve.

Plasma Osmolality and Colloid Osmotic Pressure

The clinical chemical laboratory determined plasma osmolality and plasma colloid osmotic pressure (COP) using standardized osmometry.

Histopathology

The right kidney was immediately removed at the end of the experiment and cross-sections of cortex, outer and inner medulla (n = 3 for each group) were fixed in 4% formaldehyde overnight. Tissue sections were then embedded in paraffin and 5-μm thin sections were prepared and stained with hematoxylin and eosin for histopathological analysis. Additionally, tissue sections were stained with periodic acid-Schiff to show interstitial edema formation and deposition of hydroxyethyl starch (HES).

HIF-1α Immunostaining

HIF-1α accumulation in pig kidney sections (n = 3 for each group) was assessed by immunohistochemical staining using an HIF-1α rabbit polyclonal antibody (1:150 dilution; Novus Biologicals, Littleton, CO). After deparaffinization and rehydration, the sections were subjected to antigen retrieval by cooking for 4 min in citrate buffer (pH 6.0), followed by anti–HIF-1α antibody incubation overnight at 4°C. Detection was performed using EnVision+System– horseradish peroxidase labelled antirabbit polymer (Dako, Glostrup, Denmark). Slides were washed and immunoreactivity was visualized by incubation with diaminobenzidine. Hematoxylin was used as a light nuclear counterstain. A microscope (Axioskop 40; Carl Zeiss Jena GmbH, Jena, Germany) was used for capturing images.

Experimental Protocol

At the end of surgery, Pd-porphyrin (5 mg/kg) was infused over a time period of 15 min. Then a bolus of inulin was given (20 mg/kg; I2255; Sigma Aldrich, St. Louis, MO) followed by continuous infusion (0.2 mg·kg^{-1}·min^{-1}). After 30 min baseline values were recorded. At this time point, pigs were randomized between the different experimental groups, but no attempt was made to blind investigators.

The experiments were performed in a total of 18 animals. Six animals served as sham-operated time controls and did not undergo hemodilution. One group of animals (n = 6) received colloids (Volulyte®, HES 6% 130/0.4; Fresenius Kabi, Bad Homburg, Germany), the other group (n = 6) crystalloids (Sterofundin ISO®, full electrolyte solution; B. Braun). In all pigs of the colloid group the same volume of blood/fluid (12 ml/kg) was extracted and infused (1:1), based on pilot experiments. In accordance with clinical practice, and also tested in pilots, pigs of the crystalloid group received approximately three times more volume of fluid (1:3). This resulted in hemodynamic stability in terms of cardiac output. If target hematocrit was not reached an additional 2 ml/kg of volume was exchanged (fig. 1).

Volulyte® as well as Sterofundin ISO® are commonly used for volume replacement. Both fluids are acetate-based. Details of the contents of both solutions are presented in table 1.

In all animals the hematocrit was around 30% at baseline. By extracting blood *via* the central venous catheter in the jugular vein and simultaneously giving either prewarmed colloid or crystalloid solution back *via* a peripheral vein, hemodilution was performed. H1 was reached when hematocrit was 25%, H2 at 20%, and H3 at 15%. Each step of hemodilution had a duration of 10 min for the colloid and 20 min for the crystalloid group and was followed by a stabilization period of 30 min before measurements were started. The measurements of the control group were performed at 45-min intervals. The experiment was ended after H3 measurements were taken.

Statistical Analysis

Data are presented as mean ± standard error unless indicated otherwise. Normality testing of the measured variables was performed by both visual inspection of Q-Q plots and application of the Shapiro–Wilk test (Version 20.0; SPSS, Chicago, IL). No deviations from normality were detected, justifying the use of parametric testing. The complete experimental data set, without missing data of any of the variables, was analyzed using GraphPad Prim version 6 for Mac OS X (GraphPad Software, San Diego, CA). Hypothesis testing was performed using a two-tailed test. Intragroup differences were determined by one-way ANOVA for repeated measurements, followed by Bonferroni comparison. Intergroup differences were determined by two-way ANOVA for repeated measurements, followed by Bonferroni comparison. *P* value less than 0.05 was considered significant. Values
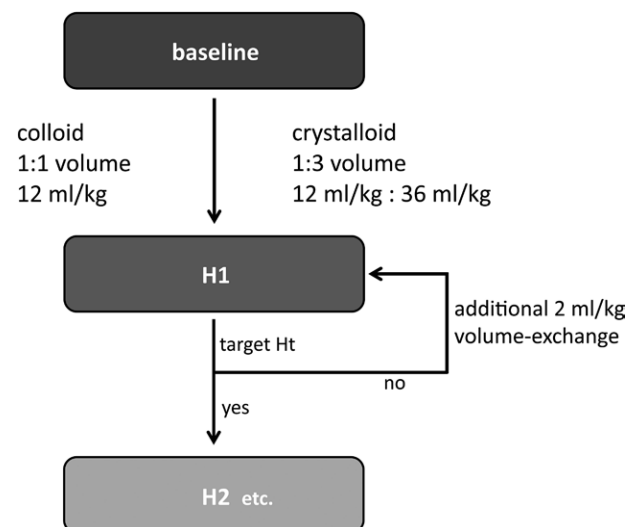

Fig. 1. Flow chart illustrating one step of acute normovolemic hemodilution. H1 = hematocrit 25%; H2 = hematocrit 20%; Ht = hematocrit.

Table 1. Contents of Fluids Used for ANH

	Volulyte®	Sterofundin ISO®
1,000 ml of solution contains		
Poly (*O*-2-hydroxyethyl) starch	60.00 g	
Molar substitution 0.38–0.45		
Mean molecular weight 130.000 Da		
Sodium acetate trihydrate, g		3.27
Sodium chloride, g	4.63	6.80
Potassium chloride, g	6.02	0.30
Magnesium chloride-hexahydrate, g	0.30	0.20
Calcium chloride-dihydrate, g	0.30	0.37
Electrolytes		
Na^+	137.0	145.0
K^+, mM	4.0	4.0
Mg^{++}, mM	1.5	1.0
Cl^-, mM	110.0	127.0
CH_3COO^-, mM	34.0	
Ca^{++}, mM		2.5
Theoretical osmolarity, mosm/l	287	309 mosm/l
Titration acidity	<2.5 mmol NaOH/l	
pH value	5.7–6.5	5.1–5.9

ANH = acute normovolemic hemodilution; ISO = isotonic.

for microvascular oxygen pressure and renal clearance were normalized to baseline.

Results

Systemic and Renal Regional Hemodynamics during ANH

MAP in the control group did not change statistically significant over the time. MAP decreased in both hemodilution groups statistically significant at H2 and H3 compared with baseline values. At H3 this decrease was statistically significant for the crystalloid compared with the control group. Heart rate remained unchanged in the crystalloid as observed in the two other groups, but was higher than in the colloid and the control group at some time points. CVP was stable in all groups at all time points. Cardiac output was not altered in the crystalloid or colloid group. In the control group, there was a statistically significant decrease at H2 and H3 compared with baseline. Stroke volume was statistically significantly decreased in the control group at all time points. At H2 there was a statistically significant difference in stroke volume between the hemodilution groups. Pulmonary capillary wedge pressure was statistically significantly lower at H1 in the control and at H2 in the colloid group compared with that at baseline. Pulmonary arterial pressure did not change in all groups at all time points. Systemic vascular resistance in the colloid group was at H2 and H3 statistically significantly lower compared with that in control. In the crystalloid group systemic vascular resistance was at H3 statistically significantly lower compared with at baseline and with that in control. RBF did not change in all groups at all time points. The ratio of RBF to cardiac output did not

show a statistically significant change in all groups at all time points. Renal vascular resistance at H3 was statistically significantly lower in the hemodilution groups compared with that in the control group. Data are presented in figure 2.

Hematocrit, Total Exchanged Volume, Kidney Weight, and Body Temperature during ANH

In the colloid group, the volume exchange was isovolemic. In the crystalloid group approximately three times more volume was infused than blood withdrawn to achieve the target hematocrit and to maintain hemodynamic stability. Because of the larger volume that needed to be exchanged in the crystalloid group, the duration of exchange was for this group twice as long. At the end of the experiment the weight of the harvest kidney was highest in the crystalloid group, but did not reach statistical significance. There was no significant statistical difference in the animal's body temperature in all groups at all time points. Data are presented in table 2.

Renal Oxygenation Parameters, and Serum Lactate Levels during ANH

Renal DO_2 showed a statistically significant decrease at H1 in the crystalloid group compared with baseline, but in the colloid group not until H2. Renal VO_2 did not show a statistically significant change in all groups at all time points. Renal oxygen extraction (O_2ER_{ren}) did not show a statistically significant change in the control group. In the crystalloid group O_2ER_{ren} showed a statistically significant increase at H2 and H3 compared with baseline and control. Renal venous partial pressure of oxygen ($P_{rv}O_2$) did not show a statistically significant change in the control group over the
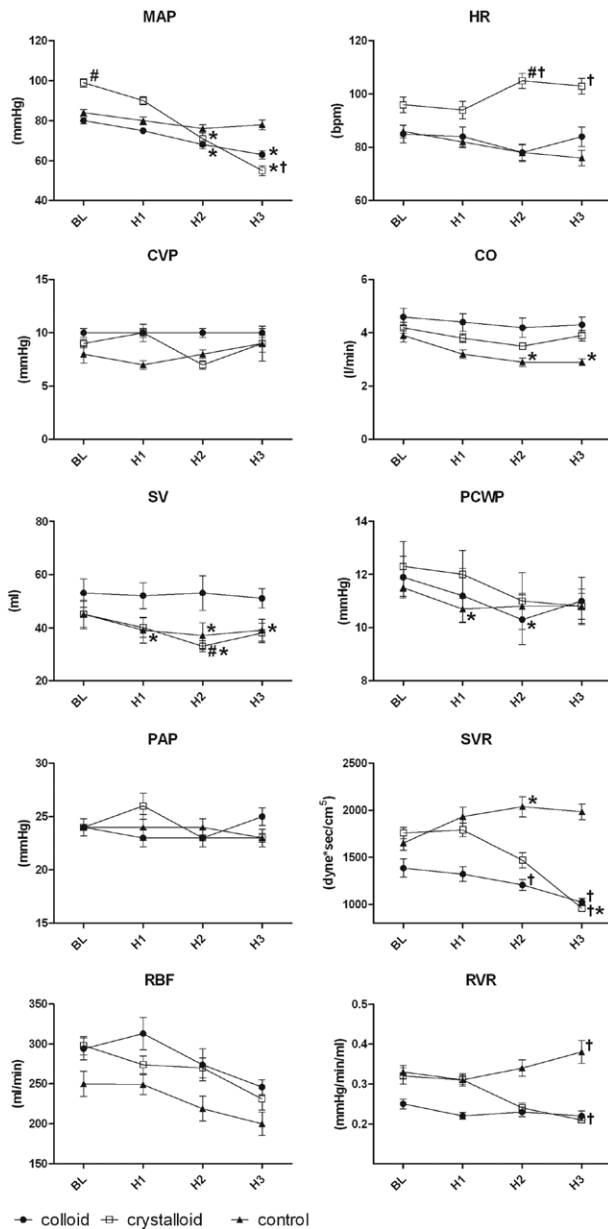
Fig. 2. Systemic and renal regional hemodynamics during acute normovolemic hemodilution. Values represent mean ± standard error. *$P < 0.05$ *versus* baseline. †$P < 0.05$ *versus* control. #$P < 0.05$ *versus* colloid. BL = baseline; CO = cardiac output; CVP = central venous pressure; H1 = hematocrit 25%; H2 = hematocrit 20%; H3 = hematocrit 15%; HR = heart rate in beats/minute (bpm); Ht = hematocrit; MAP = mean arterial blood pressure; PAP = pulmonary artery pressure; PCWP = pulmonary capillary wedge pressure; SV = stroke volume; SVR = systemic vascular resistance; RBF = renal blood flow; RVR = renal vascular resistance.

time. In the crystalloid group a drop in $P_{rv}O_2$ was statistically significant from H1 on, compared with that at baseline and in the control group. At H2 and H3 this drop was also statistically significant compared with the colloid group. $P_{rv}O_2$ showed a statistically significant decrease from baseline at H2 and H3 in the colloid group. Serum lactate levels,

as a surrogate marker of tissue hypoxia, did not show a statistically significant change over the time in the control group. At H3 there was a statistically significant increase in serum lactate (1.6 ± 0.2 mM) in the crystalloid group compared with that at baseline and in the control group. Data are presented in table 3.

Renal Microvascular Oxygenation during ANH

At baseline the cortical microvascular oxygen pressure was at average 12 ± 1 torr (1.6 ± 0.1 kPa). Cortical microvascular oxygen pressure showed a statistically significant drop in the crystalloid group compared with that in the colloid group at H3. At H3 the cortical microvascular oxygen pressure in the crystalloid group also showed a statistically significant decrease compared with that at baseline and in the control group. At baseline the outer medullary oxygen pressure was at average 21 ± 2 torr (2.8 ± 0.3 kPa). Outer medullary oxygen pressure did not show a statistically significant change in the colloid group, but showed a statistically significant decrease in the crystalloid group at H2 and H3 compared with that at baseline and H3 compared with the colloid group. At baseline the inner medullary oxygen pressure was at average 22 ± 2 torr (2.9 ± 0.3 kPa). Inner medullary oxygen pressure showed a statistically significant drop in the crystalloid group at H2 and H3 compared with the colloid group at H3 and also compared with that at baseline. In contrast, there was an increase in inner medullary oxygen pressure in the colloid group at H1 and H2 compared with that at baseline. Data are presented in figure 3.

Renal Function during ANH

At baseline the creatinine clearance was at average 21 ± 2 ml/min. Creatinine clearance stayed constant in the control and colloid group. In the crystalloid group, the creatinine clearance decreased with beginning of hemodilution. This decrease was statistically significant at H3 compared with that at baseline and that in the control group. At baseline the inulin clearance was at average 35 ± 3 ml/min. Inulin clearance in the colloid and in the control group did not show a statistically significant change at all time points. In the crystalloid group, there was a statistically significant decrease at H3 compared with that at baseline. Data are presented in figure 4.

Urinary NGAL Concentration during ANH

There was no statistically significant difference in the urinary concentration of NGAL in all groups at all time points. Dilution effects on NGAL concentration can be excluded, since the cumulative production of urine (in average 715 ± 91 ml) did not statistically significantly differ between the groups. Data are presented in figure 5.

Plasma Osmolality and COP during ANH

Osmolality statistically significantly increased in the colloid group at H3 compared with that at baseline. There were no

Table 2. Hematocrit, Total Exchanged Volume, and Kidney Weight during ANH

	BL	H1	H2	H3
Ht, %				
Colloid	30 ± 1	25 ± 1*+	20 ± 1*+	16 ± 1*+
Crystalloid	31 ± 1	25 ± 1*+	20 ± 1*+	14 ± 0*+
Control	30 ± 1	30 ± 1	31 ± 1	31 ± 1
Volume, withdrawn, ml				
Colloid		360 ± 30	370 ± 30	580 ± 100
Crystalloid		510 ± 40	580 ± 60	810 ± 110
Control				
Volume, infused, ml				
Colloid		360 ± 30	370 ± 30	580 ± 100
Crystalloid		1,430 ± 200	1,720 ± 200	2,730 ± 230
Control				
Kidney weight, g				
Colloid				102 ± 4
Crystalloid				127 ± 23
Control				84 ± 5

Values represent mean ± standard error.

*$P < 0.05$ vs. baseline. +$P < 0.05$ vs. control.

ANH = acute normovolemic hemodilution; BL = baseline; H1 = hematocrit 25%; H2 = hematocrit 20%; H3 = hematocrit 15%; Ht = hematocrit.

alterations in osmolality in the control and crystalloid group. Data are presented in figure 6.

Plasma COP showed no alteration in control group. In the colloid group there was a statistically significant increase in COP starting at H1 compared with that at baseline and that in the crystalloid group. In the crystalloid group the COP started to drop in statistically significant manner at H1 compared with that at baseline, H2, and H3 also compared with that in the control group.

Renal Histopathology and HIF-1α Immunostaining after ANH

In kidney sections of the colloid group deposits of HES could be demonstrated. Data are presented in figures 7 and 8. Renal tissue sections of the crystalloid group showed the highest formation of interstitial edema (fig. 4). HIF-1α expression was higher in the crystalloid group compared with the colloid group in which in turn HIF-1α expression was higher than in the control group and mainly localized in the distal convoluted tubules.

Discussion

This study was designed to test the hypothesis that ANH impairs renal oxygenation and kidney function in the pig. The translational framework of our study is the possible role of hemodilution in the pathogenesis of perioperative AKI, as suggested by clinical evidence.[4,5,18] Microvascular cortical oxygenation was not impaired at hematocrits of 20% or higher. However, at a hematocrit of 15% microvascular oxygenation and renal function were impaired in a statistically significant manner in the crystalloid group. This impairment of renal function was associated with formation of renal tissue edema and higher expression of HIF-1α in the distal convoluted tubules. Interestingly, there was no alteration in kidney function or microvascular oxygenation by the use of colloids for ANH.

Less fluid was necessary for reaching the required hematocrit and similar hemodynamic stability in the colloid compared with the crystalloid group, as expected from literature.[19] In the crystalloid group, at H3 MAP was statistically significantly lower compared with that at baseline and in the control group. Therefore, impairment of renal function due to a lower perfusion pressure cannot be excluded. Pilot experiments aiming at maintaining MAP by giving more fluid failed because animals frequently developed severe lung edema. For unclear reasons, MAP was at a statistically significant higher level at baseline in the crystalloid group. As all animals received adequate and comparable analgesia and sedation, a higher stress response in those animals can be excluded. Cardiac output was stable in both hemodilution groups, but isovolemic hemodilution is frequently associated with an increase in cardiac output mainly related to an increase in stroke volume.[1] In the crystalloid group a statistically significant increase in heart rate was associated with a decrease in stroke volume and therefore it cannot be excluded that these animals became hypovolemic in the course of the protocol, although pulmonary arterial pressure and CVP were stable in all groups. Alternatively, anesthesia might have influenced the cardiac output response observed during ANH in our model. Importantly, RBF did not show any statistically significant change in both hemodilution groups and therefore differences in blood supply to the kidney can be excluded. However, a higher chloride anion concentration,

Table 3. Renal Oxygenation Parameters and Serum Lactate Levels during ANH

	BL	H1	H2	H3
DO_{2ren}, ml/min				
Colloid	39 ± 5	34 ± 6	24 ± 5*	17 ± 2*
Crystalloid	40 ± 4	29 ± 2*	24 ± 3*	15 ± 3*
Control	33 ± 5	33 ± 5	29 ± 6	28 ± 5
VO_{2ren}, ml·min^{-1}·g^{-1}				
Colloid	8.2 ± 1.1	7.9 ± 0.4	6.8 ± 0.4	7.2 ± 1.0
Crystalloid	9.1 ± 2.2	9.5 ± 1.4	10.0 ± 1.4	8.4 ± 1.9
Control	7.2 ± 1.6	7.4 ± 1.1	6.8 ± 0.7	6.6 ± 1.8
O_2ER_{ren}, %				
Colloid	21 ± 2	25 ± 3	30 ± 3	45 ± 6*+
Crystalloid	22 ± 4	33 ± 4	42 ± 4*+	55 ± 7*+
Control	22 ± 2	23 ± 2	25 ± 2	23 ± 2
$P_{rv}O_2$, torr				
Colloid	51 ± 2	49 ± 2	45 ± 3*	39 ± 2*+
Crystalloid	48 ± 2	42 ± 2*+	37 ± 2*+#	32 ± 2*+#
Control	53 ± 3	51 ± 1	48 ± 2*	48 ± 2*
Serum lactate, mM				
Colloid	1.4 ± 0.2	1.0 ± 0.1	1.0 ± 0.1	1.1 ± 0.1
Crystalloid	0.9 ± 0.1	0.8 ± 0.1+	1.0 ± 0.1	1.6 ± 0.2*+
Control	1.4 ± 0.2	1.2 ± 0.2	1.2 ± 0.2	1.0 ± 0.1

Values represent mean ± standard error.

*$P < 0.05$ vs. baseline. +$P < 0.05$ vs. control. #$P < 0.05$ vs. colloid.

ANH = acute normovolemic hemodilution; BL = baseline; DO_{2ren} = oxygen delivery; H1 = hematocrit 25%; H2 = hematocrit 20%; H3 = hematocrit 15%; O_2ER_{ren} = oxygen extraction; $P_{rv}O_2$ = renal venous PO_2; VO_{2ren} = oxygen consumption.
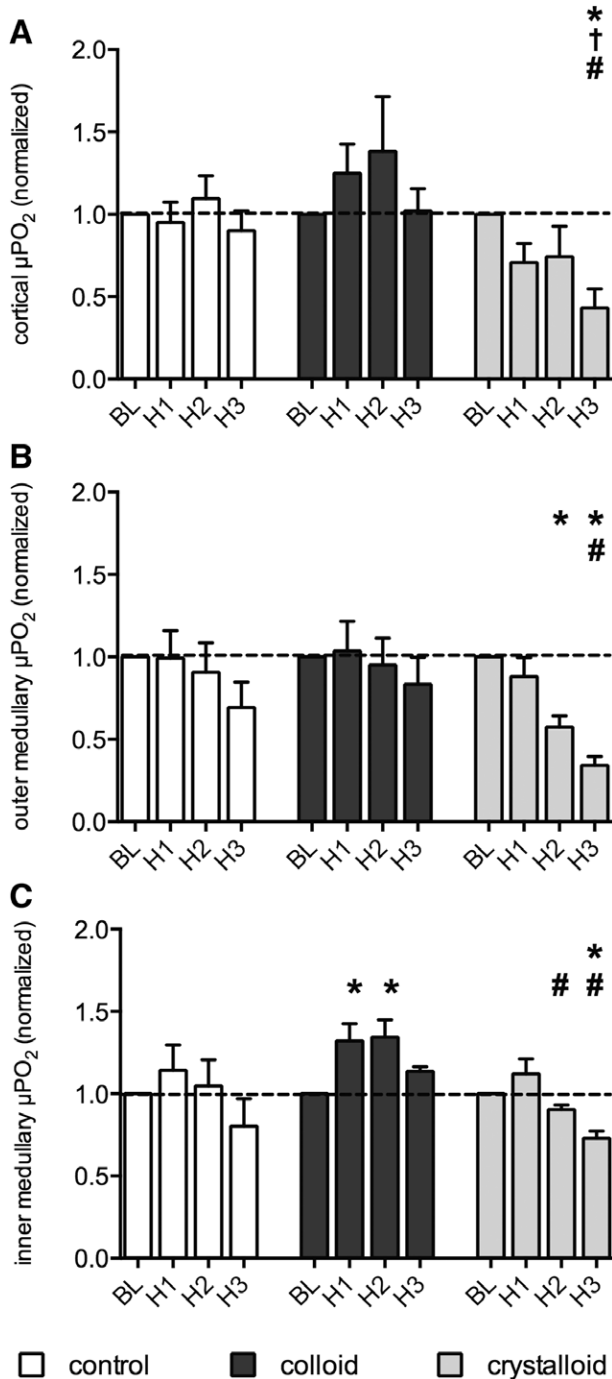
like in the crystalloid solution, can increase renal vascular resistance and decrease glomerular filtration rate.[20]

The µPo$_2$ can be regarded as being the balance between local oxygen supply and consumption at the microcirculatory level.[21] Despite the absence of statistically significant differences in DO$_2$ and VO$_2$ between both hemodilution groups, statistically significant differences in microvascular oxygenation were present. In the crystalloid group, an impaired µPo$_2$ in all kidney compartments was found on hemodilution. In this group, µPo$_2$ decreased in the renal cortex already at H1, whereas the inner and outer medullary µPo$_2$ started to decline at a hematocrit of 20%. In contrast, µPo$_2$ in the colloid group showed a statistically significant increased in the inner medulla and was not changed in a statistically significant manner in the cortex and outer medulla. Although the mechanism remains obscure, improvements in oxygenation by colloids is not exclusive for the kidney. Several studies demonstrated improved microcirculation after hemodilution with colloids.[22–24] In dogs ANH with colloids led to a more homogenous distribution of perfusion and better preservation of tissue oxygenation in skeletal muscle and liver.[25,26] One clinical study comparing different HES solutions on volunteers, showed the highest increase in tissue oxygen tensions in the quadriceps muscle undergoing ANH by the use of HES 130/0.4.[27] Improved perfusion and oxygenation in ischemic flap tissue has also been shown through ANH with HES.[28] In the current study, values of cortical µPo$_2$ of approximately 15 torr were considerably lower than
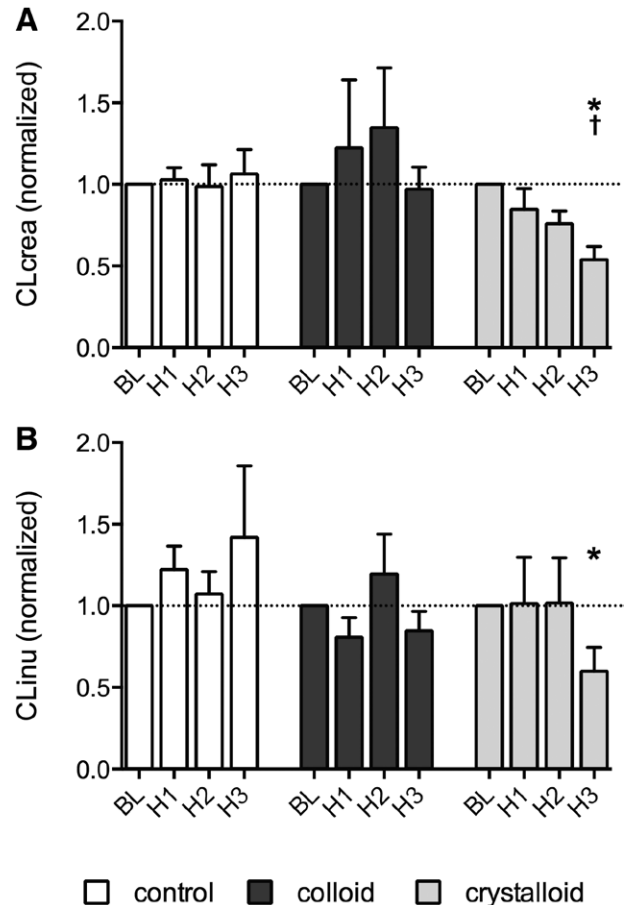
we found in previous rat experiments. However, experiments performed in rabbits using OxyLite fluorescence probes for renal cortical oxygen measurements demonstrate comparable low values.[29] Furthermore, although in general cortex tissue Po$_2$ is higher than that of the medulla, this study also found a lower cortical compared with medullary Po$_2$ which is in accordance to our observations. As previously discussed,[29] a likely explanation is that the outcome of invasive tissue Po$_2$ measurements is somewhat dependent on the thickness of measuring probes.

In comparison to our previous study in rats[7] our current observations in pigs show different results. The main differences concern renal oxygenation, with an immediate drop in microvascular oxygenation and oxygen supply dependency in an early stage of hemodilution in rats. One explanation for these contrary findings might be the different electrolyte composition of the HES solutions used. Another explanation can be the difference in baseline hematocrit with 45% in rats versus 30% in pigs. Pigs have been demonstrated to have similar physiological responses compared with humans.[12] One study on humans during hemodilution to a hematocrit as low as 15% during elective aortocoronary artery bypass surgery, showed a constant body VO$_2$.[30]

Because the deterioration of µPo$_2$ in the crystalloid group could not be explained as resulting from differences in systemic and regional renal hemodynamics, DO$_2$ and VO$_2$, the cause has to be sought within the kidney itself. In this respect, the difference in COP between crystalloids

Fig. 3. Renal microvascular oxygenation during acute normovolemic hemodilution. (*A*) Shows the cortical microvascular oxygen pressure (μPo_2). (*B*) Shows the outer medullary μPo_2. (*C*) Shows the inner medullary μPo_2. Values represent mean ± standard error. *$P < 0.05$ *versus* baseline. †$P < 0.05$ *versus* control. #$P < 0.05$ *versus* colloid. Values for μPo_2 were normalized to baseline. BL = baseline; H1 = hematocrit 25%; H2 = hematocrit 20%; H3 = hematocrit 15%.
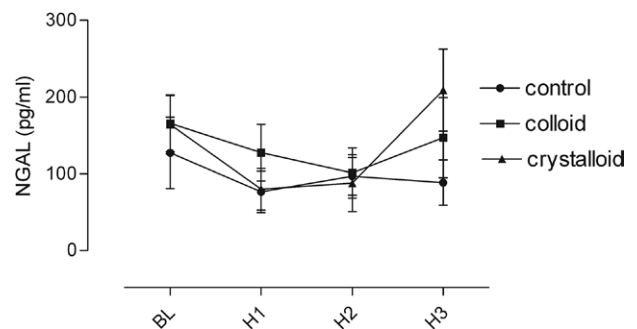
and colloids might be of importance. Furthermore, previous studies indicated that the kidney could regulate intra-RBF distribution during hemodilution.[31] The use of colloids during ANH could be associated with a redistribution of

Fig. 4. Renal function during acute normovolemic hemodilution. (*A*) Shows the creatinine clearance (CL_{crea}). (*B*) Shows the inulin clearance (CL_{inu}). Values represent mean ± standard error. *$P < 0.05$ *versus* baseline. †$P < 0.05$ *versus* control. Values for CL_{crea} and CL_{inu} were normalized to baseline. BL = baseline; H1 = hematocrit 25%; H2 = hematocrit 20%; H3 = hematocrit 15%.

intra-RBF, which might explain better tolerance to severe hemodilution in contrast to the use of crystalloids.

Early goal-directed therapy including fluid optimization improves the outcome of patients with severe sepsis

Fig. 5. Renal urinary neutrophil gelatinase-associated lipocalin (NGAL) concentration during acute normovolemic hemodilution. Values represent mean ± standard error. BL = baseline; H1 = hematocrit 25%; H2 = hematocrit 20%; H3 = hematocrit 15%.

and also patients undergoing surgery.[32–34] The goal of fluid administration is achieving adequate tissue perfusion without inducing interstitial edema.[19] Less tissue edema might be achieved by using colloids,[35,36] because hypooncotic hemodilution lowers the COP of blood.[37,38] Furthermore, a higher plasma viscosity is associated with better organ and microvascular perfusion in isovolemic hemodilution.[39] In our model COP was decreased in a statistically significant manner during hemodilution with crystalloids and increased by the use of colloids. Accordingly, the histopathological pictures showed formation of renal tissue edema in the crystalloid group. Although we did not observe an improvement in systemic hemodynamics by the use of colloids, the higher plasma viscosity might have been of benefit for the microvascular oxygenation. Tissue edema during hemodilution not only occurs in the kidney, but also in other organ systems.[40–42] Tissue edema formation leads to compression of microvessels and impaired diffusion of oxygen, which might account for the decrease in μP_{O_2} during hemodilution with crystalloids.

Are there functional consequences of hemodilution and subsequent deterioration of intrarenal oxygenation?

In the current study, inulin[43] as well as creatinine clearance showed a statistically significant decrease only in the crystalloid group at a hematocrit of 15%. In accordance with our findings, one study on dogs also detected no differences in creatinine clearance during ANH to a hematocrit of 20% with HES.[44] Rosenberger et al.[6] ascribe renal regional hypoxia a pivotal role in the development of AKI due to direct tubulotoxity. Renal cells may be injured through hypoxia itself or rather the release of reactive oxygen species.[6] The pattern of HIF in hypoxic and ischemic rat kidneys induced through anemia or carbon monoxide poisoning was different from the pattern seen after total ischemia.[45] HIF-1α was augmented around cell nuclei in the renal cortex and outer medulla, particularly in tubular segments after induced hypoxia. This emphasizes our findings, where HIF-1α is highest and mainly localized in the distal convoluted tubules in the crystalloid group. Although our study does not prove a direct association between kidney function and impaired microvascular oxygenation, our data at least demonstrate that hemodilution with crystalloids impairs renal microvascular oxygenation enough to activate cellular hypoxic adaptation. Importantly, the urinary concentration of NGAL, as a renal marker for direct
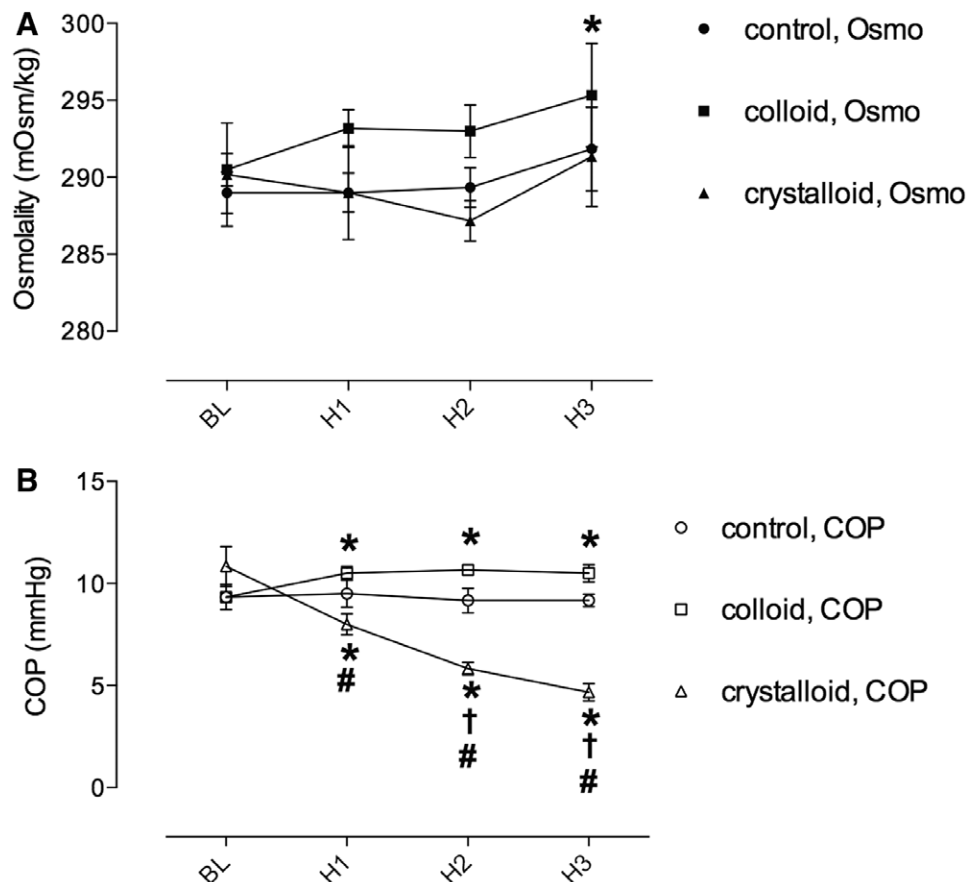

Fig. 6. Plasma osmolality and colloid osmotic pressure (COP) during acute normovolemic hemodilution. (*A*) Shows osmolality. (*B*) Shows COP. Values represent mean ± standard error. *$P < 0.05$ *versus* baseline. †$P < 0.05$ *versus* control. #$P < 0.05$ *versus* colloid. BL = baseline; H1 = hematocrit 25%; H2 = hematocrit 20%; H3 = hematocrit 15%.
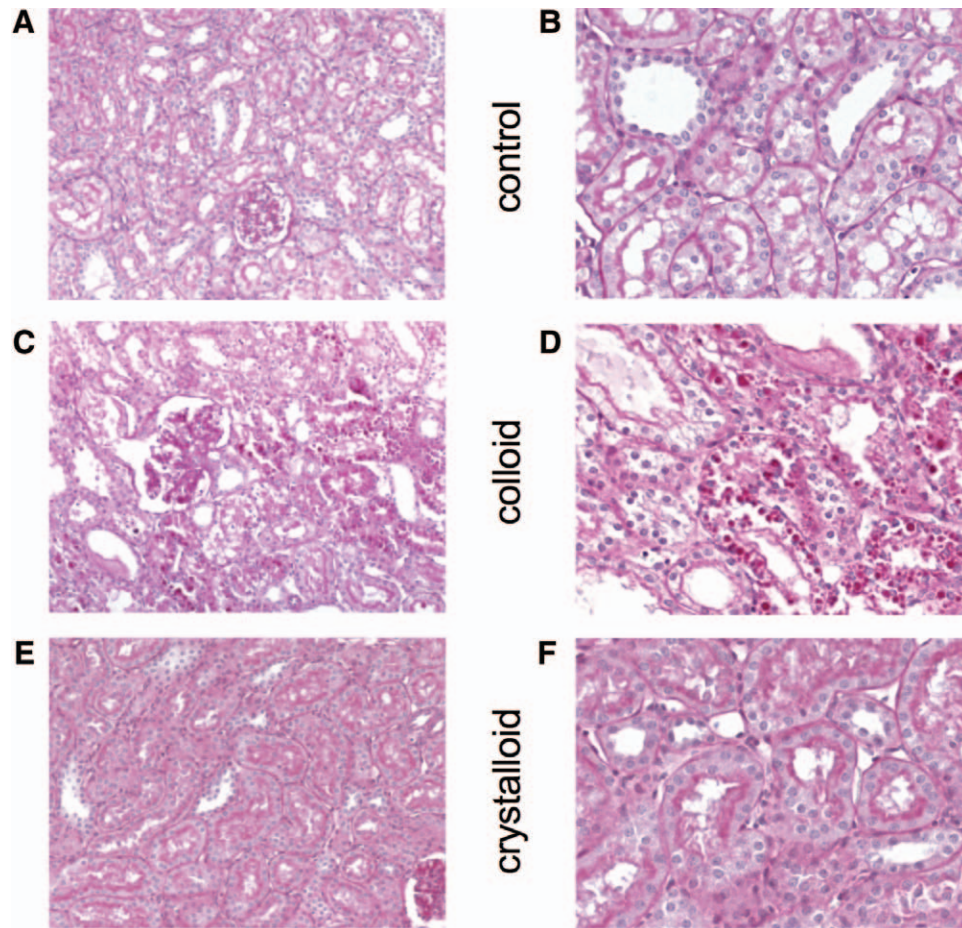
Fig. 7. Renal histopathology after acute normovolemic hemodilution. (*A* and *B*) Control group. (*C* and *D*) Colloid group. *Dark red spots* in periodic acid-Schiff–stained renal tissue indicate deposition of hydroxyethyl starch. (*E* and *F*) Crystalloid group. Sections of periodic acid-Schiff–stained renal tissue showed the highest formation of interstitial edema in the crystalloid group. Original magnification ×200 (*A*, *C*, and *E*) and ×400 (*B*, *D*, and *F*).

tubular injury,[46] did not show a statistically significant change in all groups at all time points. This indicates that renal functional loss in the crystalloid group is not due to structural damage. We hypothesize the following cascade: profound hemodilution with crystalloids reduces COP to such extent that tissue edema develops, which deteriorates microvascular function and oxygenation, leading to cellular hypoxia and metabolic adaptation, which ultimately lead to loss of function.

Controversy about whether to use crystalloid and/or colloid solutions for volume replacement in the perioperative setting exists.[19,47] In experimental studies, rapid resuscitation and improvement in tissue perfusion and oxygenation are the main advantages of colloids.[22–24] However, several randomized clinical controlled studies showed an increased risk of developing renal failure after colloid resuscitation.[48,49] Compared with our study the observational periods in these studies were much longer. Furthermore, the pathogenesis of acute renal failure in sepsis is different with low renal perfusion pressures next to capillary leakage. Early formation of renal edema in

the absence of capillary leak might be less with the use of colloids, whereas later (24–48 h) formation of edema might worsen due to the longer interstitial retention time compared with the use of crystalloids. Regarding renal risk and benefit of HES for volume replacement in nonseptic patients, results are contradictory and clear recommendations are lacking.[48] Therefore, care should be taken while directly translating our research result into clinical practice. The limitation of our study to short-term renal outcome gives our results more relevance for intraoperative normovolemic hemodilution and not fluid resuscitation in general. Accumulation of HES in the plasma or elimination of deposits is not an acute phenomenon.[50,51] Furthermore, time might have influenced our results because the experimental protocol of the crystalloid group was 30 min longer than of the colloid group. Conflicting results on renal function might also be due to the use of different HES preparations and disease patterns.[52,53] Careful reevaluation of renal safety of HES products especially in nonseptic patients will be necessary.
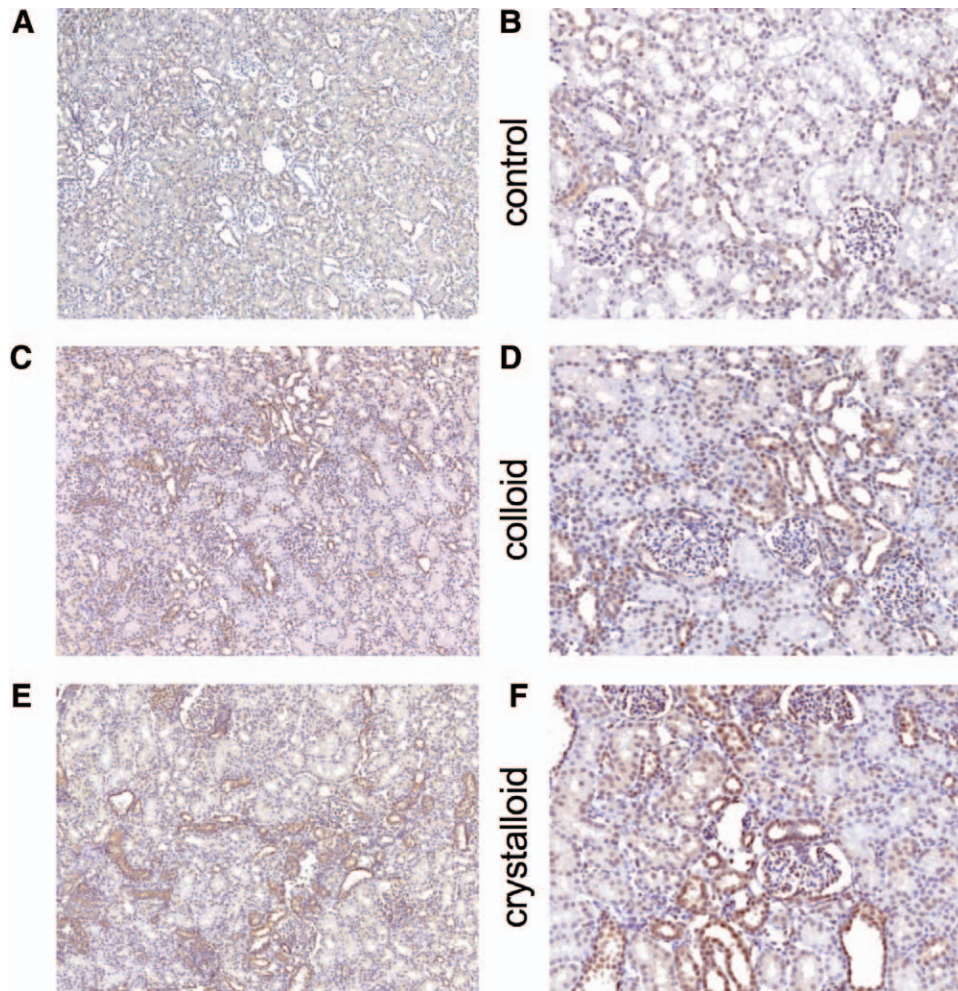
Fig. 8. Renal hypoxia-inducible factor-1α immunostaining after acute normovolemic hemodilution. Brown diaminobenzidine staining can be attributed to single hypoxia-inducible factor 1α–expressing cells in renal tissue. (*A* and *B*) Control group. (*C* and *D*) Colloid group. (*E* and *F*) Crystalloid group. Hypoxia-inducible factor-1α expression was highest in the crystalloid group and mainly localized in the distal convoluted tubules. Original magnification ×200 (*A*, *C*, and *E*) and ×400 (*B*, *D*, and *F*).

Conclusions

In our porcine model of ANH, the μPo$_2$ decreased in a statistically significant manner in all compartments of the kidney when animals are hemodiluted with crystalloids to a hematocrit of 15%. Furthermore, kidney function was impaired when using crystalloids for ANH to a hematocrit of 15%. Less tissue edema formation and an improved intrarenal microcirculation in the colloid group might account for a preserved renal microvascular oxygenation and renal function. In our study of acute hemodilution the use of colloids was superior compared with crystalloids, and our findings question the role of crystalloids as hemodilution fluid when looking at short-term functional consequences for the kidney.

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