# Renal Tissue $PO_2$ Sensing During Acute Hemodilution is Dependent on the Diluent.

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**Running Head:** Renal tissue $P_{kt}O_2$ reflects blood oxygen content following hemodilution

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## Abstract

Sensing changes in blood oxygen content ($C_aO_2$) is an important physiological role of the kidney; however, the mechanism(s) by which the kidneys sense and respond to changes in $C_aO_2$ are incompletely understood. Accurate measurements of kidney tissue oxygen tension ($P_{kt}O_2$) may increase our understanding of renal oxygen sensing mechanisms and could inform decisions regarding the optimal fluid for intravascular volume resuscitation to maintain renal perfusion. In some clinical settings, starch solution may be nephrotoxic, possibly due to inadequacy of tissue oxygen delivery. We hypothesized that hemodilution with starch colloid solutions would reduce $P_{kt}O_2$ to a more severe degree than other diluents. Anesthetized Sprague Dawley rats (n=77) were randomized to undergo hemodilution with either colloid (6% hydroxyethyl starch or 5% albumin), crystalloid (0.9% saline), or a sham procedure (control) (n=13-18 rats/group). Data was analyzed by ANOVA with significance assigned at $p<0.05$. Following hemodilution, mean arterial pressure (MAP) decreased marginally in all groups, while hemoglobin (Hb) and $C_aO_2$ decreased in proportion to the degree of hemodilution. Cardiac output was maintained in all groups after hemodilution. $P_{kt}O_2$ decreased in proportion to the reduction in Hb in all treatment groups. At comparably reduced Hb, and maintained arterial oxygen values, hemodilution with starch resulted in larger decreases in $P_{kt}O_2$ relative to animals hemodiluted with albumin or saline ($p<0.008$). Renal medullary erythropoietin (EPO) mRNA levels increased more prominently, relative to other hypoxia-regulated molecules (Glut-1, GAPDH, VEGF). Our data demonstrate that the kidney acts as a biosensor of reduced $C_aO_2$ following hemodilution and that $P_{kt}O_2$ may provide a quantitative signal for renal cellular responsiveness to

58 acute anemia. Evidence of a more severe reduction in $P_{kt}O_2$ following hemodilution with
59 starch colloid solution suggest that tissue hypoxia may contribute to starch induced
60 renal toxicity.

## Introduction

The capacity of the kidney to sense and respond to changes in hemoglobin concentration (Hb), hematocrit (Hct) and blood oxygen content ($C_aO_2$) has been demonstrated by published studies, which have assessed the impact of anemia and hypoxia on renal erythropoietin (EPO) expression (30, 45). However, the mechanism by which changes in blood oxygen content are senses *in vivo* and the biological signal(s) responsible for transducing these cellular responses have not been clearly defined. Previous studies have identified that renal cortical and medullary kidney tissue oxygen tension ($P_{kt}O_2$) are reduced following hemodilutional anemia (10, 25, 42). However, a direct proportional relationship between blood oxygen content and $P_{kt}O_2$ has not yet been clearly demonstrated. In this context, determining the impact of the degree of hemodilution and the impact of the specific non-blood resuscitation fluid is of clinical importance. For example, inadequate fluid resuscitation (38) and use of starch colloids (19, 50) have both been associated with acute kidney injury (AKI). These results place a high emphasis on selecting the most appropriate non-blood solution, and its volume, to maintain intravascular volume and renal tissue perfusion in critical care settings and during surgical procedures associated with acute blood loss.

With respect to selection of the optimal non-blood resuscitation fluid there is lack of a clear consensus regarding best clinical practice (1, 28, 40). Published studies support the following conclusions: 1) An adequate volume of intravenous crystalloid solution is required to maintain intravascular volume and renal perfusion to minimize renal injury in surgical patients (20, 38); 2) Intravascular solutions with lower chloride concentration may cause less renal injury, relative to chloride replete solutions following cardiac

surgery (7); 3) Colloid solutions may be superior to crystalloid solutions in terms of maintaining macro- and microvascular perfusion and improved clinical outcomes (2, 4, 49); 4) Starch colloid solutions are associated with adverse outcomes, including acute kidney injury, in some studies (19, 23, 37, 50); and 5) The potential benefit of utilizing albumin solutions remains controversial (15, 22, 26).

As a result, recent clinical approaches have focused on “goal directed” fluid resuscitation to improve clinical outcomes, including reducing the incidence of AKI (40, 43). However, outcomes from currently published studies have not yet provided clear answers to important clinical questions, including: 1) What is the best non-blood resuscitation fluid to optimize tissue perfusion?; 2) What is the optimal volume of fluid to maintain adequacy of intravascular volume and tissue perfusion? and 3) What are the most meaningful short and long-term clinical “goals” of intravascular fluid therapy?

Given this background, we performed acute hemodilution using different colloids (6% hydroxyethyl starch versus 5% albumin) or crystalloid (0.9% saline) solutions to determine the impact on renal perfusion and $P_{kt}O_2$. We hypothesized that hemodilution with starch colloid solutions would reduce $P_{kt}O_2$ to a more severe degree than other diluents.

## Methods

### Animal Model

All experimental protocols were approved by the Animal Care and Use Committee at St. Michael's Hospital (Toronto, ON, Canada) and conducted in accordance with Canadian Council on Animal Care and ARRIVE guidelines. Male Sprague Dawley rats (Charles River) were utilized for the experiments (n=77, 484 ± 64 g). Animals had access to food and water *ad libitum* in a housing facility with a 12:12 h light-dark cycle.

In spontaneously breathing rats, anesthesia was induced within an inhalation chamber containing 4% isoflurane in 100% oxygen. Once asleep, anesthesia was maintained via gas delivered by nose cone containing 1.5-2.0% isoflurane in 21% $O_2$. In some animals, anesthesia was maintained via nose cone for the duration of the experiments (echocardiography). In all other animals ($P_{kt}O_2$, ABG's, Co-oximetry, Kidney tissue RNA), anesthesia was delivered via a tracheostomy (14g catheter) in spontaneously breathing animals. Cannulation of the tail vein and tail artery were performed to allow infusion of the hemodilution solutions, measurement of mean arterial pressure (MAP) and drawing of arterial blood for arterial blood gases (ABG) and co-oximetry values (ABL 800 Flex, Radiometer Canada, London, ON, Canada). Heart rate was measured with electrocardiogram electrodes and the rectal temperature was monitored continuously. A thermoregulatory heating pad was used to maintain rectal temperatures near 37°C. A computerized data-acquisition system (PowerLab ADInstruments Inc., Colorado Springs CO) was used to continually monitor mean arterial pressure, heart rate (HR) and rectal temperature.

123 Animals were utilized for the following measurements: 1) $P_{kt}O_2$ and/or renal mRNA
124 measurements (n=33) and 2) Echocardiography (n=28). The total animals for each
125 experiments group include: i) Control (n=16, 478 ± 58 g); ii) 40% hemodilution with
126 saline (3:1) (n=14, 470 ± 59 g); iii) 40% hemodilution with 5% albumin (1:1) (n= 13, 502
127 ± 56 g); iv) 40% hemodilution with starch (1:1) (n= 18, 506 ± 86 g) and v) 30%
128 hemodilution with starch (1:1) (n=16, 461 ± 30 g). There was no statistical difference in
129 weight between groups (ANOVA p=0.259). Arterial blood gas and co-oximetry data
130 were collected from all groups of animals
131
132 Hemodilution Protocols
133 Rats were block randomized to 5 different protocols including: 1) time based control and
134 sham hemodiluiton; 2) hemodilution by replacing 40% of the estimated blood volume
135 (EBV) with saline in a 3:1 (fluid volume: blood volume) ratio (0.9% Sodium Chloride,
136 Baxter Corporation, Mississauga, ON, Canada); 3) 40% EBV hemodilution with 5%
137 albumin (1:1) (5% AlbuRx, CSL Behring AG, Ottawa, ON, Canada); 4) 40% EBV
138 hemodilution with hydroxyethyl starch (HES) (1:1) or 5) 30% EBV hemodilution with
139 hydroxyethyl starch (HES) (1:1) (6% Voluven 130/0.4, Fresenius Kabi, Toronto, ON,
140 Canada) (Figure 1). Blood volume was estimated to be 70 mL/kg total body weight, as
141 previously reported (12). In the case of 40% (1:1, vol:vol) hemodilution, the resultant
142 post-hemodilution Hb was nearer 70g/L as previously reported for starch hemodilution
143 (27). However, the effect of 40% (3:1, vol:vol) hemodilution with saline resulted in a Hb
144 near 90 g/L. This resulted in the inclusion of a 30% (1:1, vol:vol) starch group to allow
145 for comparison of $P_{kt}O_2$ values at comparable Hb levels. After establishing a stable

baseline, continuous measurements were initiated over the following time periods: 1) 10 minutes of baseline measurements; 2) time control or hemodilution over 10 minutes; 3) continuous measurements were performed for one hour prior to kidney harvest and animal sacrifice (isoflurane anesthetic overdose and T-61 euthanasia solution intravascular injection). Hemodilution was performed with a push-pull infusion pump (PHD2000, Harvard Apparatus, St. Laurent, QB, Canada) to maintain a steady hemodilution rate over the 10-minute period. All fluids were warmed to 37°C before administration. Cooximetry and arterial blood gases were taken into heparinized syringes at baseline, 30 and 60 minutes following hemodilution. After 1 hour following hemodilution, kidneys were harvested and kidney samples were obtained for RNA measurements.

Echocardiographic Parameters Measurement

Rats placed in the supine position underwent transthoracic echocardiography at baseline (10 minutes prior to hemodilution), 30 and 60 minutes after hemodilution, with a high-frequency ultrasound system (Vevo 2100, MS-250 transducer, Visualsonics, Toronto, ON, Canada). A researcher blinded to hemodilution treatment groups performed two dimensional long-axis images of the left ventricle in parasternal long- and short axis views with M-mode measurements at mid-papillary muscle level. Linear dimensions were analyzed offline (Vevo 2100 software v. 1.3) using the standard leading edge to leading edge technique. Three consecutive cardiac cycles were averaged for the analyses and fractional shortening (FS%), fractional area change (FAC) and cardiac output (CO) were calculated. FS% was calculated using the following

formula: (LVIDd*LVIDs)/LVIDd*100, where LVIDd and LVIDs are left ventricular end-diastolic and end-systolic internal diameters respectively. CO was calculated as the product of stroke volume (SV) and heart rate (HR). SV and ejection fraction (EF) were measured using the Teicholz cubed formula, LV volume=$7*LVID^3/(2.4+LVID)$, and the difference between ESV and EDV, where LVID, ESV and EDV are left ventricular internal diameter, end systolic volume and end diastolic volume respectively.

Kidney Tissue Oxygen Measurements ($P_{kt}O_2$)

Tissue oxygen measurements in the left kidney were performed by the phosphorescence quenching method (48) using oxygen probe Oxyphor PdG4 (14) and a time-domain OxyLED phosphorometer (Oxygen Enterprises Ltd., Philadelphia, PA). To expose the left kidney, a flank incision was made, and the subjacent soft tissue was retracted. The kidney was kept within the retroperitoneal space and in the body cavity to maintain the renal temperature comparable to the core temperature. The animal was kept in a hooded experimental chamber which prevented exposure to ambient light sources from interfering with the measurements. Oxyphor PdG4 was injected intravenously (500 μL of ~25 μM solution in saline) to reach the final concentration in the blood plasma of ~2 μM. Excitation was delivered by a laser diode ($\lambda_{max}$=635 nm), whose output was focused on the kidney surface to ~0.1 mm in diameter. The excitation light at 635 nm is relatively weakly absorbed by the endogenous tissue chromophores (e.g. hemoglobin, cytochromes etc.) (14). Nevertheless, the excitation intensity is attenuated significantly with depth. The phosphorescence of Oxyphor PdG4 ($\lambda_{max}$=813 nm) is attenuated by endogenous absorption even less than the excitation light, but the

emitted photons generated at depths have to diffuse back to the surface to be sensed by the detector. As a result, the volume of tissue probed in such a surface excitation/emission scheme does not have strictly defined borders, and yet based on the light transport coefficients at the wavelengths specified (3) it is reasonable to assume that the recorded signals are dominated by the phosphorescence originating in the upper 1-3 mm of tissue with tissue layers closer to the surface giving larger contribution to the signal. Therefore, the measurements performed in this study carry information primarily about microvascular $P_{kt}O_2$ within the renal cortex and superficial medullary tissue weighted by microvascular density within each of the compartments. Such values are reflective of $P_{kt}O_2$ within the first 3 mm of depth within the kidney, as previously determined by microelectrode analysis (34) and assessment of microvascular $pO_2$ as previously published (24, 42, 46, 47). The laser pulse was modulated to generate rectangular 10 μs-long pulses, which were followed by 2 ms-long phosphorescence acquisition periods. The emission detecting light guide was positioned 1-2 mm directly above the focused area of excitation and received input from the tissue at a wavelength of 813 nm. Two hundred data collection cycles were averaged in a single measurement (2 ms), and the measurements were performed at 5s intervals. Experimental conditions were deemed acceptable with a signal to noise ratio of phosphorescence decay of greater than 2.

Renal Tissue RNA Isolation

Samples of renal tissue were taken from the superficial renal cortex (1-2 mm depth) and also from the deeper cortex and outer medulla (2-5 mm depth). RNA isolation and quantitative PCR (qPCR) were performed by a scientist blinded from the treatment groups. mRNA levels of erythropoietin (EPO), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), vascular endothelial growth factor (VEGF) and glucose transporter 1 (GLUT1) were measured in tissue from the right kidney. The right kidney was extracted and divided into superficial cortex or combined deep cortex and superficial medulla. The samples were immediately frozen in liquid nitrogen and stored at -80°C until analysis. Total RNA was isolated from kidney tissues using Trizol reagent (Invitrogen, USA) according to the manufacturer's protocol and RNA was quantified by NanoDrop ND-2000 Spectrophotometer (Thermo Scientific, USA). Two µg of total RNA was used for reverse transcription to cDNA using a High Capacity cDNA Reverse Transcription Kit (ABI Applied Biosystems, USA) following manufacturer's protocol. cDNA was amplified by qPCR (QuantStudio 7 Flex, Applied Biosystems) using PowerUp SYBR Green Master Mix (ABI Applied Biosystems, USA). A list of primers used for qPCR can be found in Table 4, RPL13a was used as the housekeeping gene.

Sample Size Calculation and Statistical analysis

The sample size was calculated for the primary outcome of change in $P_{kt}O_2$ based on data from previous experiments (35, 42, 47). We estimated a $P_{kt}O_2$ difference between 40% saline and 40% starch of 10mmHg with a standard deviation of 6.0, power of 0.8 and an alpha of 0.05. Our predicted sample size was 7 for each group. Normality was tested with the Shapiro-Wilk test. For data that demonstrated normal distribution

(arterial blood gas, echocardiogram outcomes, and kidney $P_{kt}O_2$ measurements) analyses were performed utilizing a Two-Way ANOVA with repeated measures and post-hoc Tukey test for pairwise comparison. Data for “changes from baseline” in $P_{kt}O_2$, MAP and temperature failed the Shapiro-Wilk test and analysis was performed with Kruskal-Wallis one-way ANOVA on ranks. Post-hoc pairwise comparisons were tested with Dunn’s Method. Comparison of regressions was performed with One-Way ANCOVA. mRNA outcomes were compared by a One-Way ANOVA on Ranks and post hoc Dunn’s method, using Sigmaplot software (Systat Software Inc., San Jose, CA). ($p<0.05$ was taken to be significant).

## Results

### Physiological Parameters

Mean arterial pressure (MAP) values were not different amongst groups at baseline. A transient drop in MAP was observed immediately following hemodilution in all treatment groups (Figure 2A, $p<0.03$). After 30 minutes, the MAP of 40% saline and 30% starch groups remained reduced, relative to controls (Figure 2A, $p<0.03$). There were no significant differences in MAP post-hemodilution between treatment groups.

At baseline, rectal temperatures were maintained near 37°C no significant differences were observed amongst groups. Rectal temperatures tended to drop during the hemodilution period in some groups (30% and 40% starch and 40% saline) but were restored to values comparable to baseline 30 minutes following hemodilution (Figure 2B, $p<0.04$).

### Arterial Blood Gas (ABG) Analysis Following Hemodilution

The baseline ABG and cooximetry data were not different amongst groups at baseline. All values remained stable over time in the control group (Table 1 and 2). Following hemodilution, there were no statistically significant differences in pH, $p_aO2$, $p_aCO2$, or $S_aO_2$ or lactate levels, over time or amongst groups (Table 1). By contrast, there was a statistically significant decrease in $HCO_3$ after hemodilution with saline (21.2 ± 2.1 mmol/L) relative to all groups (~24 mmol/L)($p< 0.02$) (Table 1). This was accompanied by an increase in $Cl^-$ level (117.9 ± 9.4 mmol/L) following hemodilution with saline, relative to baseline (105.9 ± 94.6 mmol/L) ($p<0.05$) (Table 2).

Following hemodilution, the hemoglobin concentration (Hb) and blood oxygen content ($C_aO_2$) decrease significantly in all groups (Table 1). The magnitude of the decrease in hemoglobin and blood oxygen content were greatest for 40% hemodilution with albumin and starch (post-hemodilution Hb~70 g/L and $C_aO_2$~95 ml/L, respectively). The reduction in Hb was smaller in the 40% saline and 30% starch groups (post-hemodilution Hb ~90 g/L and $C_aO_2$~115ml/L, respectively) (Table 1, Figure 3B, $p<0.005$, $p<0.013$, respectively).

$P_{kt}O_2$ Values

At baseline, the $P_{kt}O_2$ values were not significantly different between groups with an overall mean value of 62.5 ± 6.2 mmHg. The $P_{kt}O_2$ values in the time based control group remained stable for the duration of the experiment. In all hemodilution groups, there was a significant reduction in $P_{kt}O_2$ initiating with hemodilution and being sustained at 30 minutes post hemodilution (2-way ANOVA time effect $p<0.001$) (Figure 3A). At the predetermined assessment time of 30 minutes post hemodilution, there were significant group and interaction effects (2-way ANOVA $p<0.001$ for both). At the 30 minutes post hemodilution, the $P_{kt}O_2$ was lowest in the 40% starch hemodilution group (34.1 ± 6.6 mmHg, $p<0.001$ relative to baseline). Post-hoc between group analysis demonstrated a statistical difference at 30 minutes between the $P_{kt}O_2$ of the 40% starch hemodilution group and the 40% saline hemodilution group (46.9 ±5.9 mmHg)(Figure 3A; $p<0.02$).

Comparisons of the mean values of $P_{kt}O_2$ relative to blood oxygen content ($C_aO_2$) were assessed at baseline and 30 minutes post hemodilution (Figure 3B). As expected, no

292 significant differences were observed at baseline or over time in the control, non-
293 hemodiluted animals. Hemodilution with saline (40% 3:1 v/v) and starch (30% 1:1 v/v)
294 resulted in similar levels of blood oxygen content reduction ($C_aO_2$, 115.9 ± 18.3 and
295 114.5 ± 8.1 ml/L, respectively). Hemodilution with starch (40% 1:1 v/v) or albumin (40%
296 1:1 v/v) resulted in lower blood oxygen contents ($C_aO_2$, 93.9 ± 9.7 ml/L and 96.7 ± 7.5
297 ml/L, respectively). In both cases, the $P_{kt}O_2$ tended to be lower for the starch
298 hemodilution groups, relative to saline and albumin, at comparable blood oxygen
299 content levels (Figure 3B).
300
301 Physiological Data and $P_{kt}O_2$ Present as Changes from Baseline
302 Transient reductions in both MAP and rectal temperatures were noted immediately post
303 hemodilution (Fig 4A, 4B). However, these normalized after 30 minutes, and no
304 significant differences amongst groups were observed at this time. The *a priori* primary
305 outcome for this study was the change in $P_{kt}O_2$ 30 minutes post hemodilution between
306 groups (Figure 5). The data indicate that the largest change in $P_{kt}O_2$ occurred in the
307 40% starch hemodilution group. The change in kidney $P_{kt}O_2$ value in the 40% (-26.3 ±
308 6.5 mmHg) and 30% (-18.8 ± 3.5 mmHg) starch groups were significantly different
309 relative to controls (1-way ANOVA on ranks $p<0.05$). These data indicate that the
310 largest changes in $P_{kt}O_2$ were in the starch hemodilution groups at two different levels.
311 Analysis of change in $P_{kt}O_2$ relative to change in blood oxygen content demonstrated a
312 positive correlation when analyzed by 1-way ANCOVA (Figure 6). An analysis was
313 performed comparing all data for starch colloid hemodilution with non-starch colloid
314 hemodilution (i.e. saline and albumin) in order to assess the potential impact of starch

315 on $P_{kt}O_2$. In this analysis, we observed a significant linear relationship between change
316 in $C_aO_2$ and $P_{kt}O_2$ for both comparisons. However the slope for the starch hemodilution
317 [$P_{kt}O_2 = 0.610 + (0.333 * C_aO_2)$] was steeper for starch relative to non-starch solutions
318 [$P_{kt}O_2 = 0.287 + (0.222 * C_aO_2)$] indicating a more profound drop in $P_{kt}O_2$ for comparable
319 change in blood $C_aO_2$ content (ANCOVA, $p< 0.009$).
320
321 Renal Expression of Hypoxia Sensitive mRNA Levels
322 Hypoxia inducible factors (HIF)-dependent mRNA levels were measured in the
323 superficial cortex (<2mm depth) and in the combined inner cortex and outer medulla
324 (~2-5mm depth) (EPO, VEGF, GAPDH and GLUT1). There were no significant
325 differences between mRNA levels of VEGF and GAPDH in either tissue location
326 between groups (Figure 7). Lower expression of GLUT1 mRNA was observed in the
327 40% starch hemodilution relative to albumin (Figure 7, $p<0.05$). By contrast, in the deep
328 cortical and outer medullary region we observed a higher level of EPO mRNA
329 expression in the saline and 40% starch groups ($p<0.05$), relative to other experimental
330 groups. There were no changes in expression of other hypoxia inducible mRNA species
331 in the deep cortical and superficial medullary tissue.
332
333 Echocardiographic Parameters Following Hemodilution
334 There were no significant changes in heart rate, end diastolic volume, end systolic
335 volume, left ventricle mass, ejection fraction and fractional shortening at any time or
336 amongst groups (Table 3, Figure 8). Thirty minutes after hemodilution, there was a
337 significant decrease in cardiac output in the 40% saline group ($p<0.05$ relative to

baseline) and a significant increase in cardiac output in the 40% albumin group ($p<0.05$ relative to baseline and saline group) (Figure 8, Table 3). After 60 minutes, there were no statistically significant differences between cardiac output relative to baseline or amongst the different groups.

Relationship between Left Ventricular End Diastolic Volume and Cardiac Output Following Hemodilution

To assess the relationship between left ventricular end diastolic volume (LVEDV) (preload) and CO (Frank Starling Curve) (33), we utilized experimental data from the current study comparing 40% hemodilution with 1:1 starch to assess the relationship between LVEDV and CO. The curve demonstrating a best fit through these data points suggests that the animals in our hemodilution studies existed at a linear, preload-dependent portion of the Frank Starling Curve, demonstrating responsiveness of cardiac output to LVEDV (Figure 9, $r^2=0.43$).

## Discussion

Important novel findings of this study include that the kidney is extremely sensitive to changes in hemoglobin concentration and blood oxygen content as reflected by directly proportional changes in $P_{kt}O_2$ following hemodilution. A clear linear relationship was observed between the change in blood oxygen content and $P_{kt}O_2$ at decreasing hemoglobin levels from ~140-70 g/L. This reflects the capacity of the kidney to translate changes in blood oxygen content (hemoglobin concentration and hematocrit) into changes in oxygen tension within the kidney tissue ($P_{kt}O_2$). Specific cells within the kidney can then respond to this change in tissue $P_{kt}O_2$ as evident by the observed changes in EPO RNA expression. The observed changes in $P_{kt}O_2$ likely reflect measurements from the superficial renal cortex as our light based measurement technique records microvascular $pO_2$ values predominantly from the first 2 mm of depth within the kidney. However, the finding that EPO mRNA levels are increased in deeper tissue sections (2-5 mm in depth) containing tissue from the outer renal medulla suggests that renal tissue oxygen tension was reduced both within the renal cortex and renal medulla. It also demonstrates that the anemia induced renal tissue hypoxia is sensed by specific cells capable of upregulating EPO mRNA expression in the kidney (13). Of interest, the EPO response was much more sensitive to hemodilution than that of other HIF-dependent molecules including GLUT1, GAPDH and VEGF as previously observed (46, 47). Our data are consistent with the known physiological function of the kidney, which allows it to respond in a very sensitive manner to changes in Hb (13, 30, 45) following acute hemodilution (47).

The relative impact of hemodilution with different crystalloid and colloid solutions demonstrates the renal response is significantly influenced by different diluents. Of importance, hemodilution with starch (30 or 40% estimated blood volume) caused a more severe reduction in $P_{kt}O_2$ at comparable decreases in hemoglobin concentrations relative to saline or albumin colloid. The steeper slope of the blood oxygen content-$P_{kt}O_2$ relationship suggests that starch colloid negatively impacts microvascular kidney perfusion to a greater degree than do saline (3:1) or 5% albumin (1:1). These data suggest a potential hypoxic mechanism for the observation that starch colloid are associated with renal toxicity in critically ill patients (50) and those undergoing fluid resuscitation following surgical procedures (19). The mechanism(s) by which starch impairs renal oxygen delivery and predisposes to renal injury might include both macrovascular (venous congestion) (6) and/or microvascular components (glyocalyx disruption) (29). Further research will be required to elucidate the specific mechanism.

Our data suggests that hemodilution with albumin results in preferential maintenance of renal perfusion and tissue oxygen tension. In rats hemodiluted with albumin, the cardiac output was significantly elevated relative to baseline after 30 minutes. At this time point, renal tissue $P_{kt}O_2$ was higher than in any other hemodilution group. In addition, we did not detect an elevated level of EPO mRNA following hemodilution with albumin. This data is consistent with a previously published translational study assessing the impact of traumatic brain injury, hemorrhage and fluid resuscitation in rats (5). In this study, data demonstrated that fluid resuscitation with albumin preferentially improved brain tissue oxygen tension and electrophysiological function in hippocampal slices. While

398 previously published clinical research do not demonstrate a clear benefit of albumin
399 resuscitation, further research will be needed to determine if albumin is preferential for
400 fluid resuscitation under specific clinical conditions (11, 15, 36, 44).
401
402 Our echocardiographic data clearly demonstrated a linear relationship between end
403 diastolic volume (preload) and cardiac output as predicted by the Frank-Starling Law.
404 However, no clear correlation was observed between cardiac output and $P_{kt}O_2$. For
405 example, fluid resuscitation with saline resulted in a transient decrease in cardiac output
406 which recovered to baseline levels after 30 minutes, the $P_{kt}O_2$ levels for this group were
407 comparable to that following 40% hemodilution with albumin in which the cardiac output
408 increased at 30 minutes. By contrast, the cardiac output was maintained following 40%
409 hemodilution with starch; however, this exposure resulted in the lowest level of $P_{kt}O_2$.
410 The lack of observed relationship between cardiac output and $P_{kt}O_2$ is of importance
411 because clinical studies have utilized this non-invasive measurement of cardiac output
412 as a measurement of responsiveness to fluid boluses to determine the optimal level of
413 fluid resuscitation to promote tissue perfusion (19, 20, 40, 43). This approach is based
414 on the assumption that increased cardiac output will result in increased tissue perfusion.
415 Clinical trials have demonstrated that adequacy of intravascular fluid resuscitation is
416 required to maintain renal function (20, 38). However, our data demonstrate that
417 changes in cardiac output do not necessarily correlate directly with renal tissue
418 perfusion suggesting that additional clinical tools are required to directly assess and
419 measure microvascular tissue perfusion in critically ill patients. Such an approach has
420 been performed utilizing a fiber optic luminescent optode to measure urine $pO_2$

following cardiac surgery (51) and a Clark type electrode to measure brain $P_tO_2$ following neurotrauma (39). In both cases, low $P_tO_2$ correlated with adverse outcome and organ injury. With respect to brain $P_tO_2$, algorithms directed at optimizing brain $P_tO_2$ have shown promise in a phase 2 trial to improve clinical outcomes (39). In summary, assessment of tissue oxygen tension can inform clinical practice and may improve patient outcomes.

The Kidney as a Biosensor of Reduced Oxygen Content

From a physiological perspective, the kidney has been subdivided into different functional components including the renal cortex (glomerular filtration and proximal tubule free absorption) and the deeper cortical and medullar structures which are involved with maintaining solute gradients for passive and active electrolyte and nutrient absorption. From the perspective of oxygen, which readily diffuses between tissues (16), there is a continuous gradient, with a higher $P_{kt}O_2$ in the renal cortex and a progressively decreasing level of $P_{kt}O_2$ within the deep cortex and medullary structures as exemplified in experimental studies (25, 34, 35, 42). This suggests that there is a continuum in $P_{kt}O_2$ which is relatively higher in the superficial renal cortex and decreases progressively with increased tissue depth within the renal medulla (10, 25, 35, 42). In addition, studies that measure both renal cortical and medullary $P_{kt}O_2$ by different methods demonstrate that both values tend to change in parallel in response to stimuli, which include cardiopulmonary bypass and acute hemodilution (9, 10, 25, 32). In the current study, we used a combination of intravascular oxyphor to measure renal tissue oxygen tension in superficial regions that included the outer renal cortex through

444 to the superficial renal medulla. These measurements demonstrate a relationship
445 between reduced blood oxygen content and decreased renal cortical $P_{kt}O_2$ and cellular
446 hypoxia responses in the renal medulla (erythropoietin RNA) that suggest the presence
447 of renal tissue hypoxia sensing in response to initiated changes in blood oxygen
448 content.
449
450 From the molecular perspective, our data demonstrates that EPO is among the most
451 sensitive HIF-responsive molecules within the kidney during acute hemodilution. This is
452 consistent with previous results from previously published data in a study utilizing a
453 model of moderate anemia (hemoglobin ~90g/L) in which renal EPO gene expression
454 far exceeded (~25x) that observed in the brain (~1x increase)(35). These data
455 demonstrate that the EPO responsiveness in the kidney may be a primary hypoxic
456 response to anemia (30, 35, 45). This sensitive EPO response has been identified
457 following acute anemia (8, 30, 35, 46), hemorrhage resuscitation (45), atmospheric
458 hypoxia (13, 45) and molecular conditions that mimic hypoxia (31). Data form these
459 studies demonstrate that the distribution of EPO producing cells changes from being
460 predominantly in the deep renal cortex and superficial medulla, at baseline, to becoming
461 more broadly distributed throughout the renal cortex and medullary tissue under hypoxic
462 conditions (13, 31). The increase in EPO producing cells and EPO transcription
463 observed in these studies, results in a rapid increase in renally secreted EPO (41) to a
464 degree that is proportional to the level of anemia (30, 47), hemorrhage (13, 45) and
465 hypoxia (13, 45). Thus, EPO production is regulated at the cellular and transcriptional
466 level. Preliminary clinical studies have suggested that both renal medullary (urinary)

hypoxia and systemic EPO levels may correlate with acute kidney injury after hemodilutional anemia during cardiopulmonary bypass (21, 51). However, further work is needed to establish whether these potential biomarkers of renal hypoxia can be used to guide clinical care and prevent acute renal injury.

We utilized $P_{kt}O_2$ as a composite measure that would include aspects of renal oxygen supply and demand. Previous assessment of renal blood flow in rats hemodiluted with starch solution, or rendered anemic after treatment with a RBC antibody, did not demonstrate any acute changes in renal blood flow (RBF) measured by ultrasound Doppler at Hb values near 90 and 70 g/L (35, 47). As a result, renal blood flow was not measured in the current study. By contrast, animals with vagal and renal denervation undergoing acute volume expansion with saline or albumin solutions demonstrate an increase in RBF and reduced renal vascular resistance with volume expansion (17, 18). This effect was not observed when volume expansion was performed with whole blood suggesting that changes in Hct may have contributed. Future studies should address the contribution of changes in renal vascular resistance and blood flow (renal $O_2$ supply) and the impact of changes in renal tubular sodium excretion (renal $O_2$ demand), as contributors to $P_{kt}O_2$.

Limitations

There are many limitations in our current study; 1) we did not assess multiple levels of hemodilution or early changes in $P_{kt}O_2$ with mild hemodilution and therefore cannot comment on early renal responses to mild reductions in blood oxygen content; 2) we

compared hemodilution to two levels of blood oxygen content at hemoglobin near 70 and 90 g/L but we could not correct for other parameters associated with saline versus colloid resuscitation; 3) hemodilution with balanced crystalloid solutions ( i.e. Normasol, Ringer's lactate) was not tested and hemodilution with these solutions may have different impacts on renal perfusion. Saline was utilized in this study to control for comparable NaCl levels as both albumin and starch solutions are maintained in normal saline; 4) we were unable to assess the determinants of renal oxygen consumption, renal oxygen delivery and tubular sodium reabsorption; and 5) measurements of hypoxia inducible factor (HIF) protein were not performed in this study; 6) we did not perform any long term assessment of renal function and therefore cannot comment on whether any long term renal dysfunction may occur.

Perspectives and Significance:

The experimental data presented demonstrate a clear linear relationship between hemoglobin concentration, hematocrit and blood oxygen content and microvascular $P_{kt}O_2$ in rats undergoing acute hemodilution; thereby emphasizing the role of the kidney as a biosensor of tissue hypoxia during acute hemodilutional anemia. Reduced $P_{kt}O_2$ following hemodilution resulted in a preferential increase in EPO mRNA levels, relative to other hypoxia response elements, suggesting that the EPO response is a sensitive and important component of the renal response to anemia-induced changes in $P_{kt}O_2$. Changes in microvascular $P_{kt}O_2$ did not correlate with acute changes in cardiac output demonstrating dissociation between macrovascular and microvascular measures of perfusion. Finally, hemodilution with starch colloid resulted in lower $P_{kt}O_2$ levels, relative

513 to values observed after hemodilution with albumin or saline, at comparable Hb
514 concentrations, suggesting that inhibition of microvascular tissue oxygenation may
515 contribute to renal toxicity associated with starch colloids (19, 50).
516
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528

**Figure 1**. A flowchart of our experimental groups and procedures is presented. After anesthesia and vascular access G4 oxyphor probe was injected IV (500μL of 25μM Oxyphor PdG4) and stable baseline parameters were established. An initial arterial sample provided baseline arterial blood gas (ABG) and cooximetry values. Hemodilution was then performed over ten minutes. Two post-hemodilution blood samples were taken immediately after hemodilution and at 30 minutes. Echocardiographic assessment was performed at indicated time points. Measurement of mean arterial pressure, heart rate, rectal temperature and $P_{kt}O_2$ were performed for 30 minutes prior to sacrificing anesthetized animals and harvesting kidney tissue at 60 minutes.

**Figure 2**. Effect of acute hemodilution on mean arterial pressure (MAP) and rectal temperature in rats hemodiluted with different fluids (n=33). **A**: At baseline, the MAP was not different between groups. Immediately following hemodilution all treated rats had a marginally lower MAP relative to time-based controls (#; $p<0.03$). After 30 minutes of recovery, MAPs were not different from baseline measurements or control animals in the 40% albumin and 40% starch hemodilution groups. By contrast, the MAP remained lower in the 40% saline and 30% starch groups, relative to controls (ǂ; $p<0.03$). **B**: At baseline, the rectal temperature was not different between groups, being near 37$^{o}$C in all cases. Immediately following hemodilution there was a transient decrease in rectal temperature in all groups relative to baseline (ǂ; $p<0.04$ for all). Thirty minutes following hemodilution there were no significant differences in temperature between treatment groups. All data are presented as means ± standard deviation, significance was determined by two-way ANOVA.

553

554 **Figure 3. A**: Kidney tissue oxygen tension ($P_{kt}O_2$) was maintained in control animals
555 over time. There was a significant decrease in $P_{kt}O_2$ in all treatment groups immediately
556 following hemodilution and at 30 minutes (#; $p<0.001$ versus control). At 30 minutes
557 post hemodilution the $P_{kt}O_2$ of the 40% saline group was significantly higher than that of
558 the 40% starch group ($; $p<0.02$ between groups). **B**: There was an expected reduction
559 in the blood oxygen carrying capacity ($C_aO_2$) in all hemodiluted groups at 30 minutes
560 post hemodilution (*; $p<0.001$). The $C_aO_2$ reduced to values near 115 mL/L for the 40%
561 saline and 30% starch groups. There was a further reduction in $C_aO_2$ to near 95 ml/L for
562 the 40% starch and 40% albumin groups (‡; $p<0.012$). The resultant $P_{kt}O_2$ values were
563 lower in all treatment groups following hemodilution (§; $p<0.001$). There was a
564 statistically significant difference between the mean $P_{kt}O_2$ of the 40% starch group from
565 that of the 40% saline ($; $p<0.02$). All data are presented as means ± standard
566 deviation, significance was determined by two-way ANOVA.

567

568 **Figure 4.** Changes in mean arterial pressure (MAP) and temperature between baseline,
569 immediately post hemodilution and 30 minutes post hemodilution. **A**: The MAP
570 decreased transiently in the 40% saline and 30% starch groups (#; $p<0.05$), relative to
571 controls, immediately post hemodilution. There were no significant differences between
572 groups at 30 minutes post hemodilution. **B**: The rectal temperature decreased
573 transiently in the 40% saline group (#; $p<0.05$), relative to controls, immediately post
574 hemodilution. However, there were no significant differences at 30 minutes post

hemodilution. All data are presented as medians and quartiles, significance was determined by one-way ANOVA on ranks.

**Figure 5.** Changes in kidney tissue oxygen tension ($P_{kt}O_2$) between baseline, immediately post hemodilution and 30 minutes post hemodilution. Immediately following hemodilution, the $P_{kt}O_2$ of 40% saline and 40% starch groups decreased compared to controls (#; $p<0.05$). At 30 minutes post-hemodilution, the change in $P_{kt}O_2$ for the 40% starch and 30% starch groups were both significantly lower compared to controls (#; $p<0.05$). All data are presented as medians and quartiles, significance was determined by one-way ANOVA on ranks.

**Figure 6.** For comparison, individual values for change in $P_{kt}O_2$ and $C_aO_2$ were assessed in the combined non-starch groups (40% albumin and 40% saline) and comparted to values for the combined starch groups (30% and 40%). Changes in oxygen capacity ($C_aO_2$) were plotted against changes in kidney tissue oxygen tension ($P_{kt}O_2$) and assessed by linear regression. The regression line for the starch group had a steeper slope relative to the non-starch group [Starch regression line: $P_{kt}O_2 = 0.61+(0.333* C_aO_2)$ vs. non-starch regression line is $P_{kt}O_2=0.287+(0.222* C_aO_2)$; $p<0.009$ by ANCOVA]. Regressions and 95% confidence intervals are shown.

**Figure 7.** Fold changes in mRNA samples from the superficial cortex or combined deep cortex and superficial medulla of the rat kidneys. **A**: No significant difference was observed between the mRNA fold changes of glyceraldehyde 3-phosphate

dehydrogenase (GAPDH) between treatment groups or between tissues. **B**: Glucose transporter 1(GLUT1) fold change was significantly lower in the superficial cortex of 40% starch treated animals compared to 40% albumin ($; $p<0.05$). **C**: No significant differences in mRNA fold change of vascular endothelial growth factor (VEGF) between treatment groups or between tissues. **D**: Erythropoietin (EPO) mRNA fold change was significantly higher in the combined deep cortex, superficial medulla of both 40% saline and 40% starch treatment groups compared to control (#; $p<0.05$). All data are presented as medians and quartiles, significance was determined by two-way ANOVA on ranks.

**Figure 8**. Echocardiogram data from rats. **A**: No significant difference in heart rate was observed between groups. **B**: No significant difference in left ventricular end diastolic volume was observed between groups. **C**: No significant difference in stroke volume was observed between groups. **D**: Thirty minute after hemodilution, the cardiac output was increased in the 40% albumin group (*; $p<0.05$) and significantly decreased in the saline group relative to baseline (*; $p<0.05$). The cardiac output values of these two groups were significantly different at 30 minutes (#; $p<0.05$). By 60 minutes, there were no differences in cardiac output observed between groups. All data are presented as medians and quartiles, significance was determined by two-way ANOVA on ranks.

**Figure 9**. The cardiac output shows a linear relationship to left ventricular end diastolic volume (LVEDV). This is representative of the early linear portion of the Frank Starling curve ($r^2 = 0.43$).

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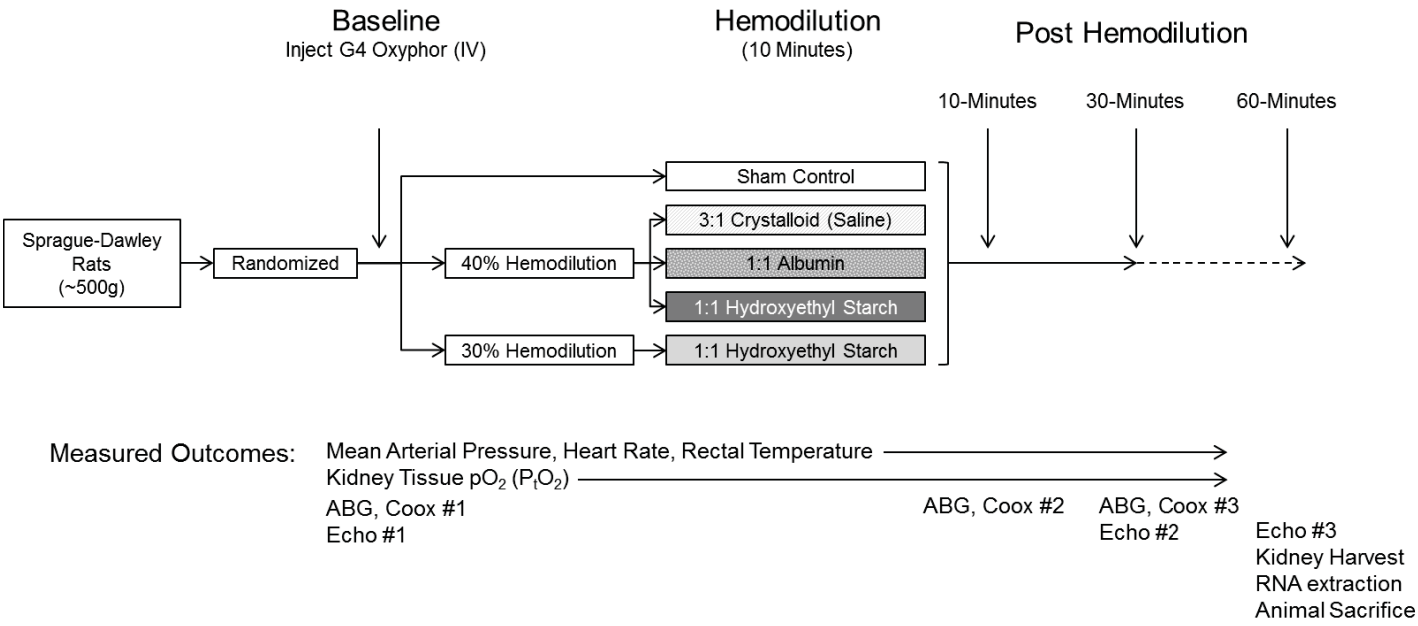

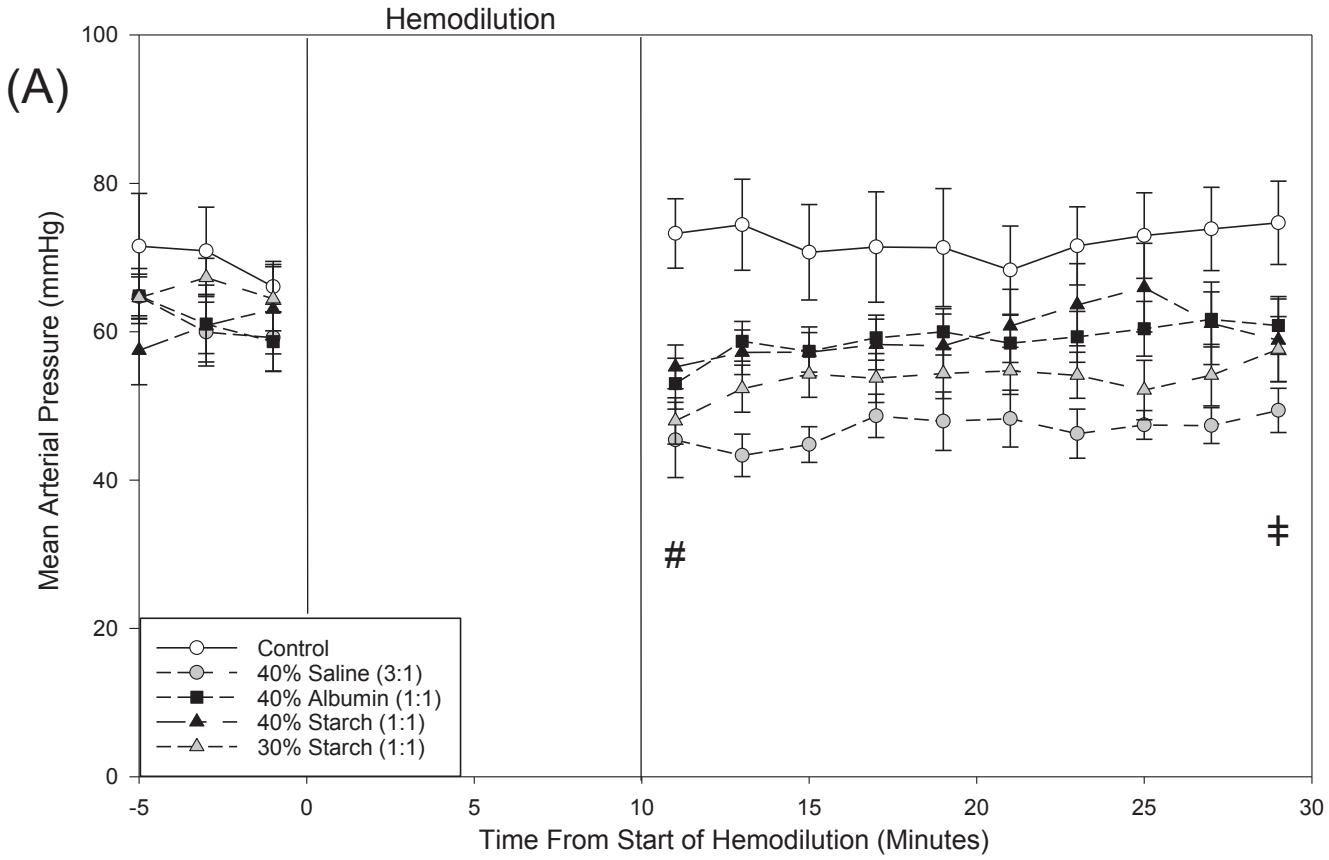


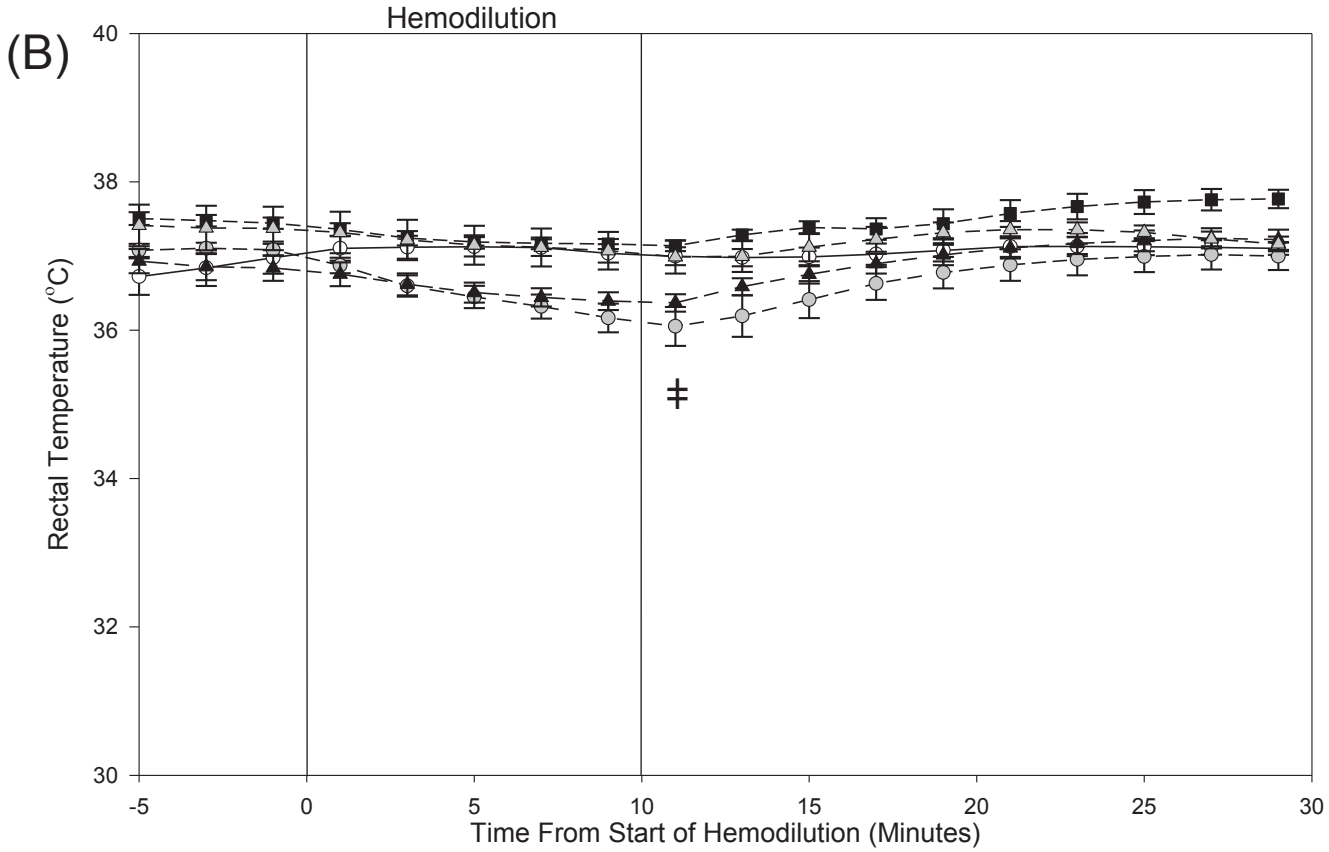

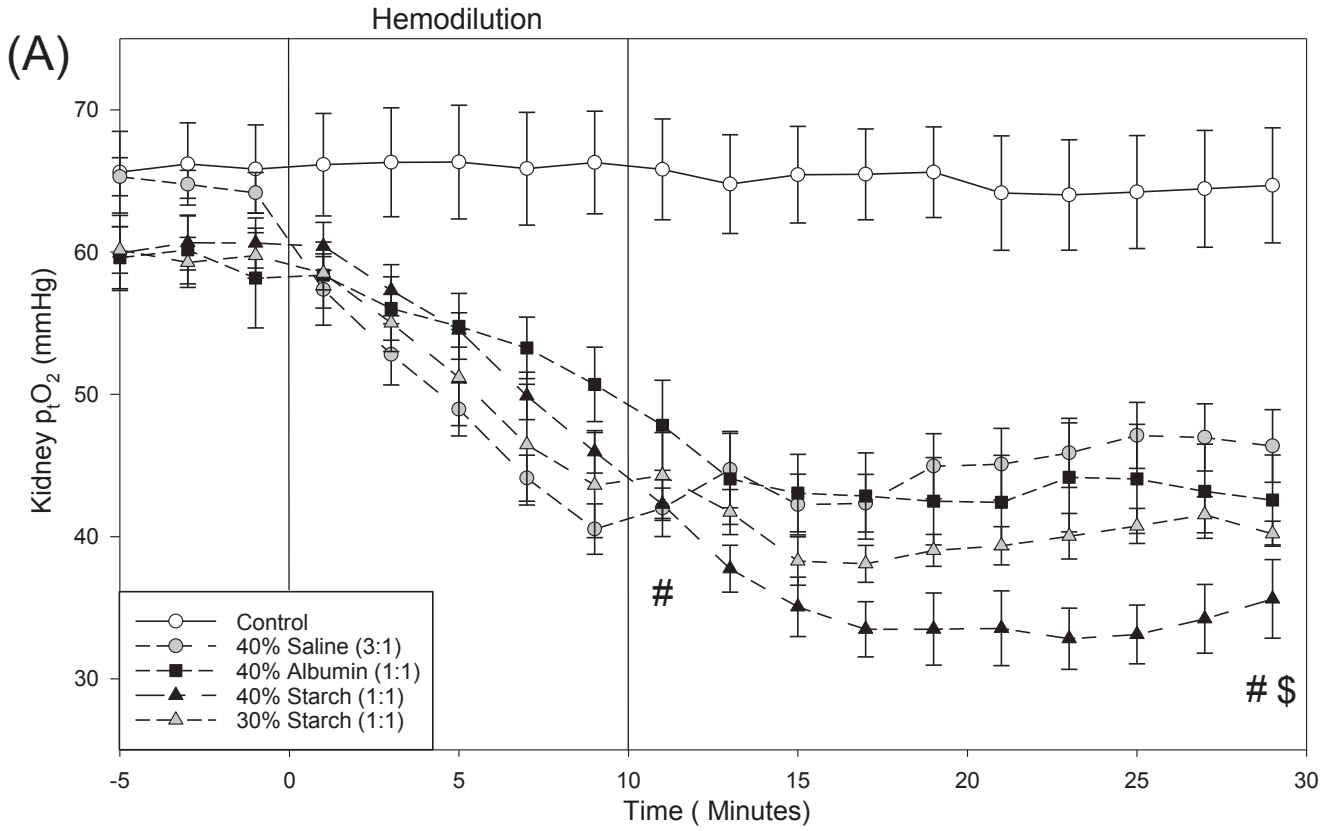


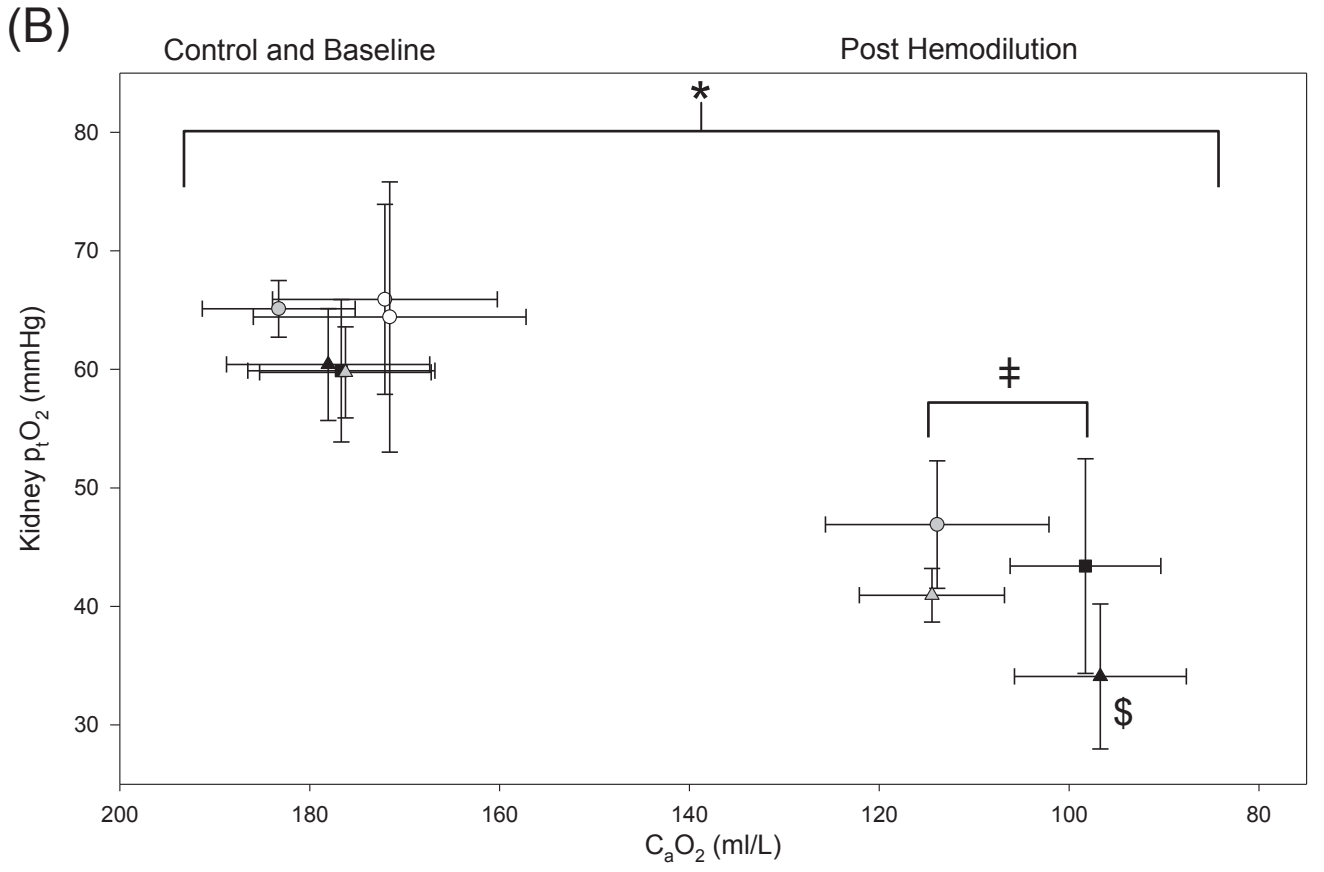

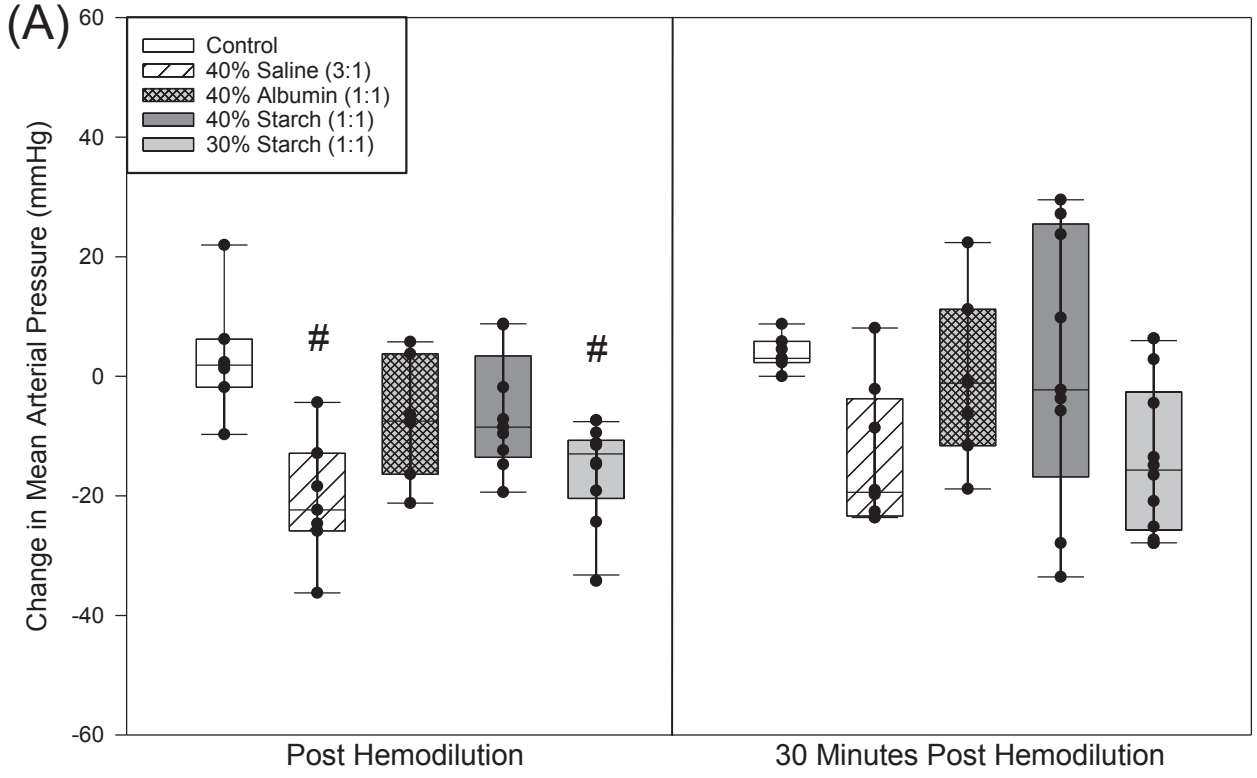


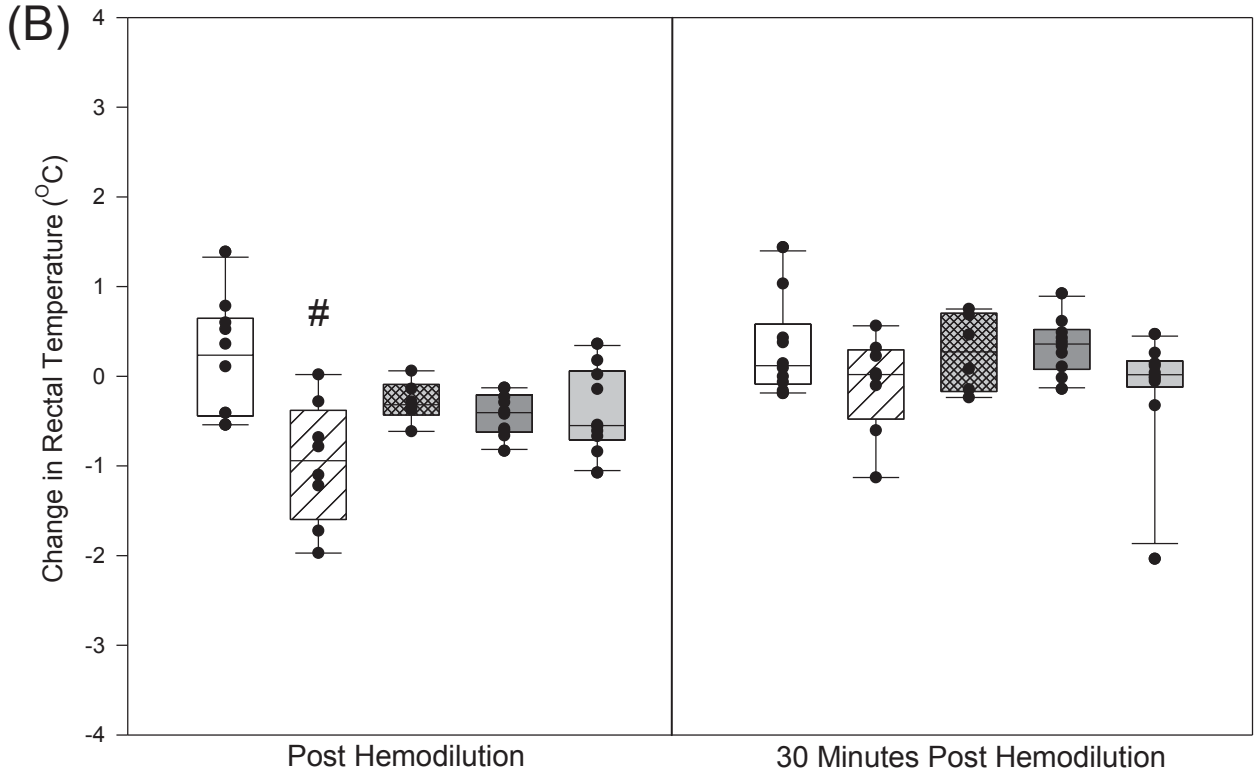

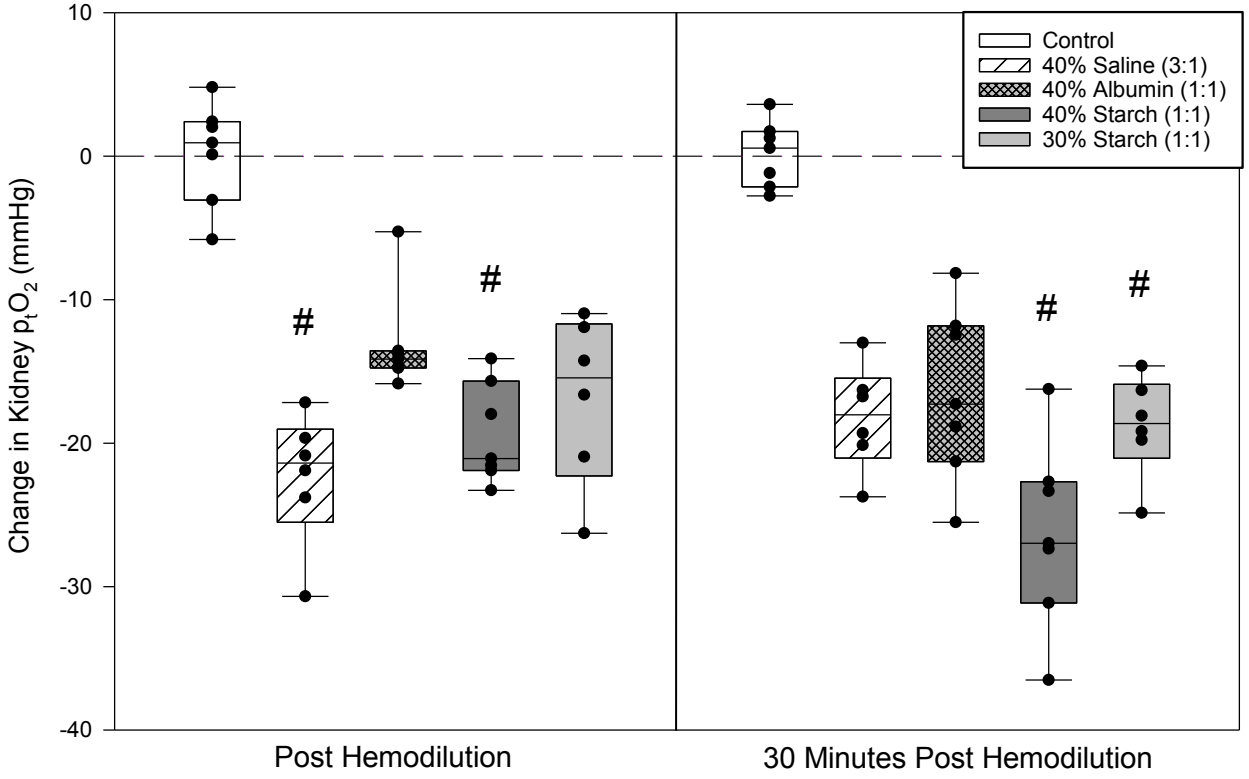

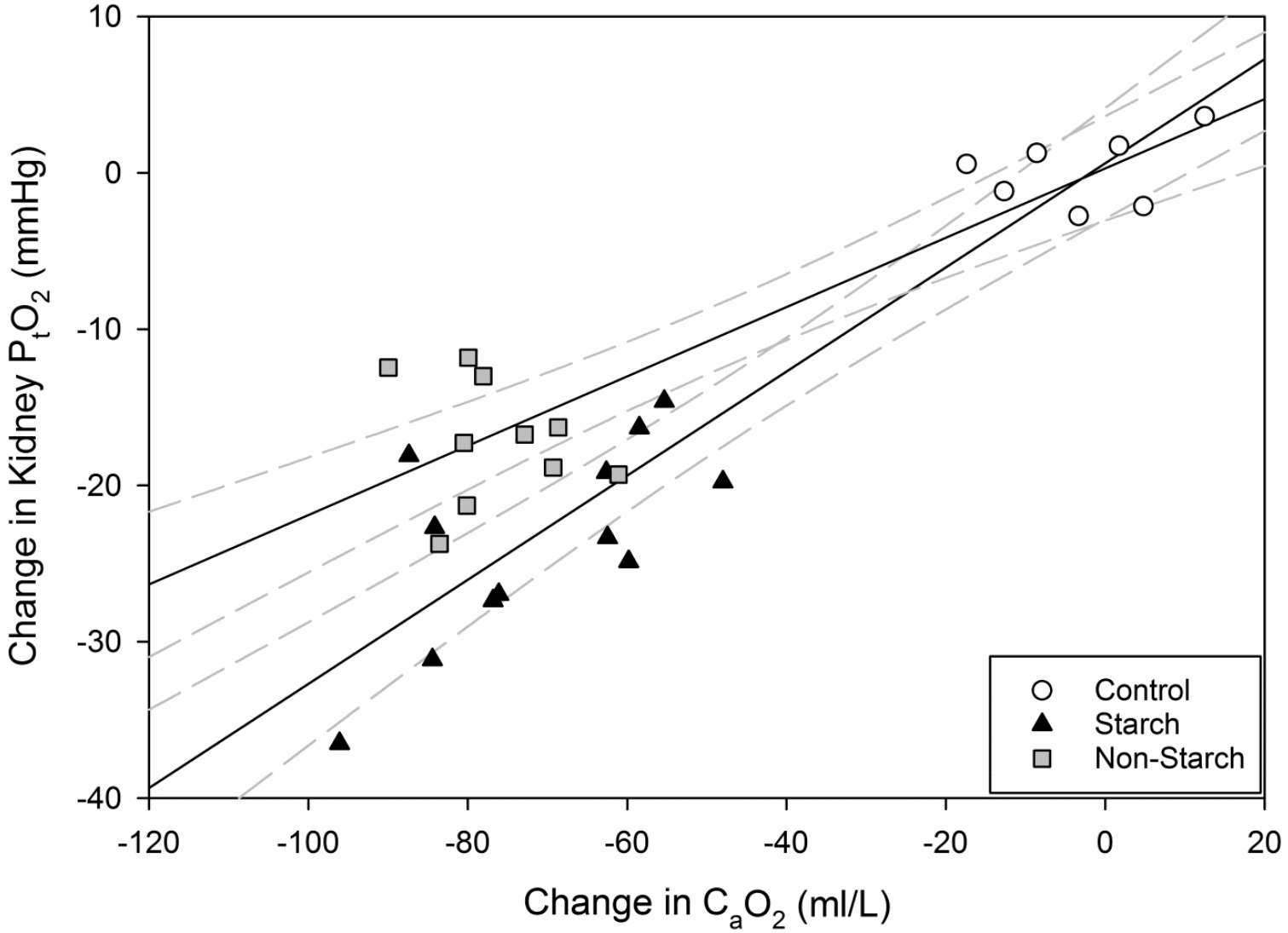

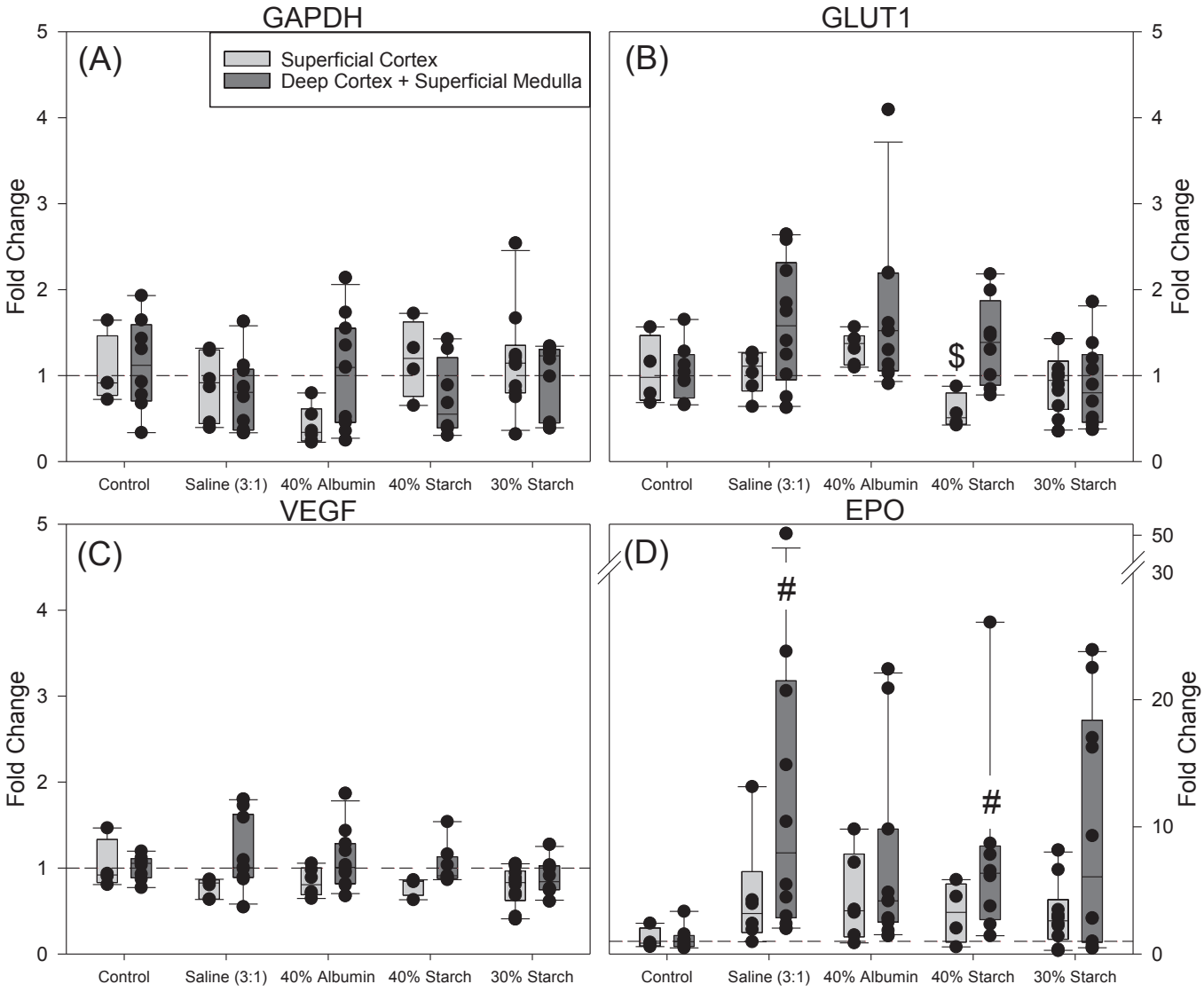

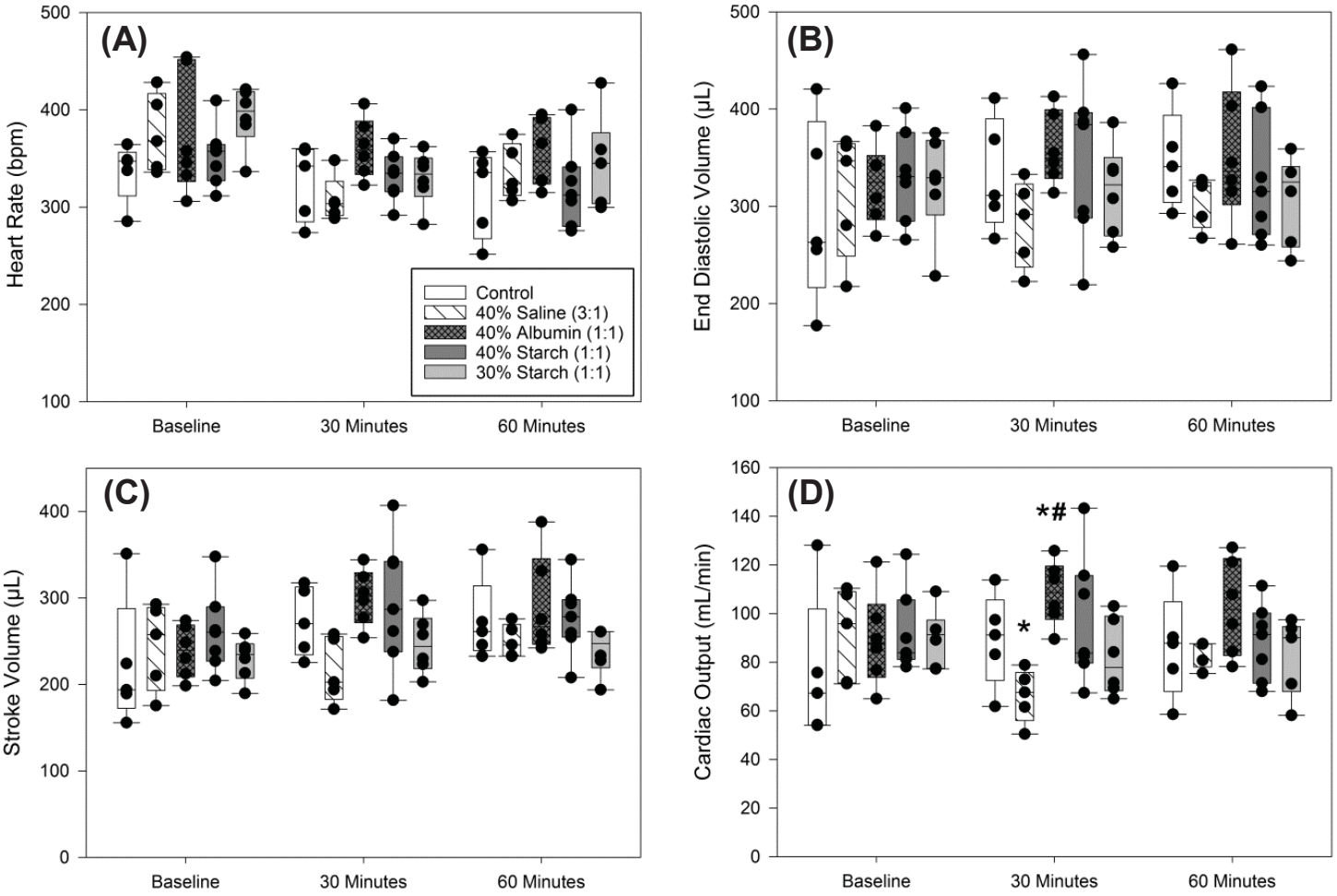

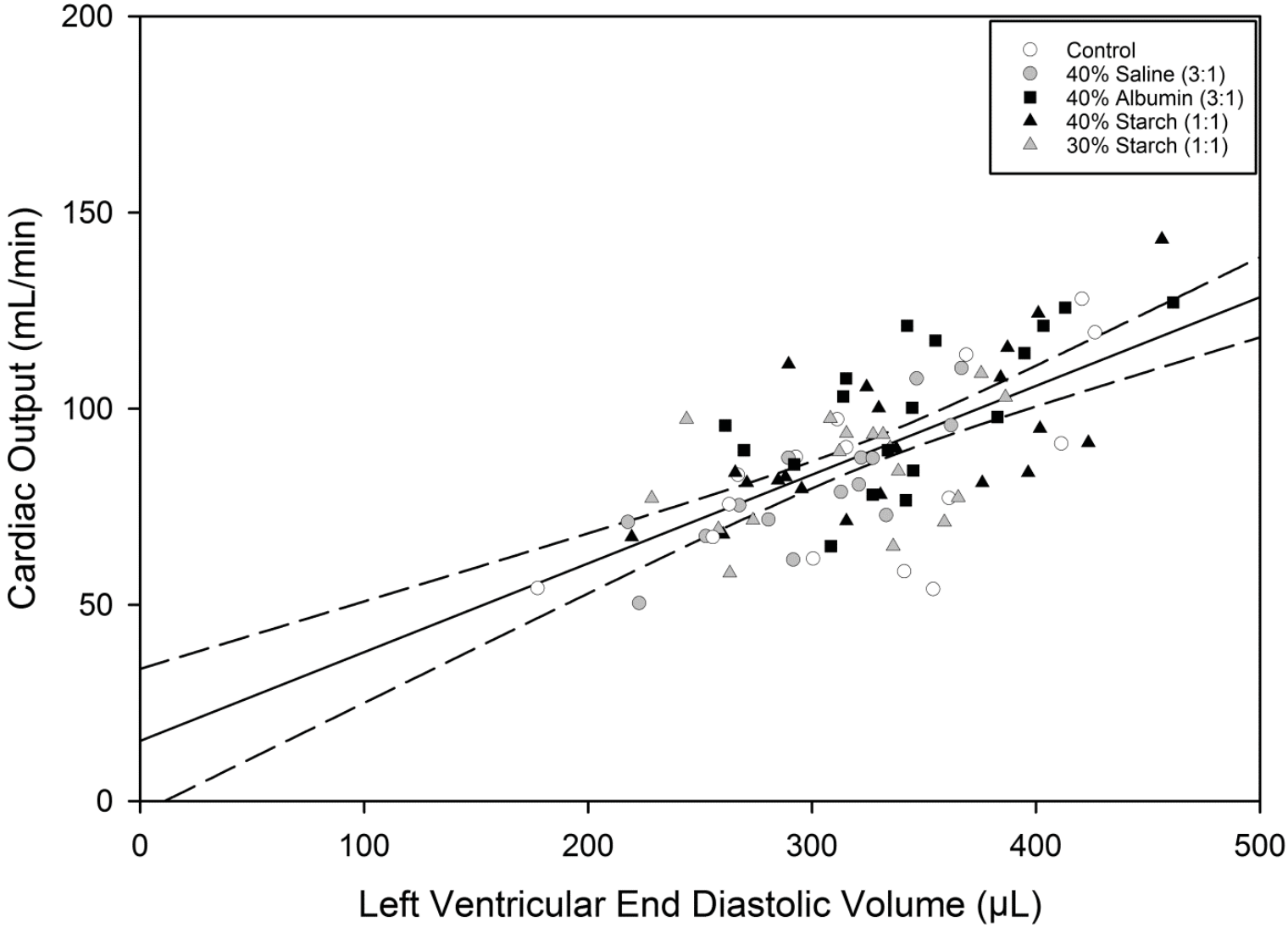

Table 1. Arterial blood gas and cooximetry data.

| | Hemodilution | pH | pCO2 (mmHg) | pO2 (mmHg) | cHCO3 (mmol/L) | ctHb (g/L) | sO2 (%) | CaO2 (ml/L) |
|---|---|---|---|---|---|---|---|---|
| Baseline | Control | 7.39 ± 0.03 | 43.1 ± 4.6 | 103.2 ± 14.2 | 24.8 ± 1.9 | 133.0 ± 8.4 | 93.6 ± 2.2 | 173.3 ± 11.3 |
| | 40% Saline (3:1) | 7.36 ± 0.04 | 47.4 ± 7.0 | 110.8 ± 16.7 | 24.8 ± 2.0 | 138.2 ± 6.1 | 93.0 ± 4.5 | 178.9 ± 11.8 |
| | 40% Albumin (1:1) | 7.38 ± 0.03 | 45.3 ± 3.5 | 108.3 ± 14.4 | 25.1 ± 1.2 | 133.5 ± 10.4 | 93.3 ± 3.2 | 173.3 ± 14.8 |
| | 40% Starch (1:1) | 7.39 ± 0.02 | 42.9 ± 2.6 | 106.2 ± 16.5 | 25.2 ± 1.0 | 136.1 ± 8.5 | 92.4 ± 3.3 | 175.0 ± 12.8 |
| | 30% Starch (1:1) | 7.38 ± 0.02 | 44.3 ± 3.2 | 95.8 ± 4.3 | 25.2 ± 1.1 | 135.9 ± 6.8 | 93.2 ± 1.2 | 176.3 ± 9.5 |
| 30 Min. | Control | 7.38 ± 0.05 | 44.2 ± 9.8 | 103.2 ± 23.5 | 24.3 ± 0.9$^{\$}$ | 133.1 ± 6.2 | 90.7 ± 7.1 | 168.0 ± 14.9 |
| | 40% Saline (3:1) | 7.32 ± 0.03 | 43.3 ± 6.3 | 101.9 ± 13.4 | 21.2 ± 2.1* | 90.1 ± 16.5$^{\#}$ | 92.7 ± 2.3 | 115.9 ± 18.3$^{*\#}$ |
| | 40% Albumin (1:1) | 7.36 ± 0.04 | 43.3 ± 5.4 | 109.4 ± 15.0 | 24.6 ± 3.5$^{\$}$ | 71.0 ± 9.4$^{\#\$‡}$ | 94.3 ± 2.4 | 96.7 ± 7.5$^{*\#\$‡}$ |
| | 40% Starch (1:1) | 7.38 ± 0.04 | 42.6 ± 5.5 | 104.3 ± 17.9 | 24.5 ± 1.1$^{\$}$ | 71.1 ± 6.3$^{\#\$‡}$ | 94.0 ± 3.2 | 93.2 ± 9.1$^{*\#\$‡}$ |
| | 30% Starch (1:1) | 7.37 ± 0.01 | 41.0 ± 5.7 | 109.0 ± 3.5 | 23.6 ± 1.7$^{\$}$ | 87.4 ± 6.7$^{\#}$ | 94.2 ± 1.0 | 114.5 ± 8.1$^{*\#}$ |
| 60 Min. | Control | 7.35 ± 0.05 | 46.2 ± 10.0 | 104.8 ± 18.9 | 23.8 ± 0.7 | 125.1 ± 5.9 | 92.5 ± 5.5 | 161.1 ± 11.1 |
| | 40% Saline (3:1) | 7.33 ± 0.04 | 42.0 ± 6.3 | 112.4 ± 14.2 | 21.3 ± 1.7 | 86.4 ± 8.4 | 93.9 ± 2.5 | 112.8 ± 9.9 |
| | 40% Albumin (1:1) | 7.36 ± 0.04 | 43.0 ± 5.1 | 111.7 ± 13.4 | 24.8 ± 3.4 | 69.5 ± 8.7 | 94.8 ± 1.4 | 94.9 ± 6.1 |
| | 40% Starch (1:1) | 7.38 ± 0.05 | 43.1 ± 7.2 | 110.1 ± 15.5 | 24.3 ± 1.0 | 74.0 ± 9.6 | 95.0 ± 1.8 | 98.0 ± 13.0 |
| | 30% Starch (1:1) | 7.38 ± 0.03 | 42.4 ± 3.0 | 112.4 ± 3.8 | 24.3 ± 1.1 | 90.7 ± 5.1 | 94.1 ± 1.4 | 118.8 ± 6.9 |

*; p<0.05 vs Baseline. #; p<0.05 vs Control. $; p<0.05 vs Saline 3:1. ‡; p<0.05 vs Starch 30%.

Table 2. Arterial blood electrolyte data.

| | Hemodilution | cNa+ (mmol/L) | cK+ (mmol/L) | cCl- (mmol/L) | cCa2+ (mmol/L) | cGlu (mmol/L) | cLac (mmol/L) |
|---|---|---|---|---|---|---|---|
| Baseline | Control | 138.9 ± 2.7 | 4.7 ± 0.5 | 107.2 ± 4.5 | 1.3 ± 0.3 | 13.9 ± 3.0 | 1.8 ± 0.5 |
| | 40% Saline (3:1) | 137.2 ± 3.1 | 4.8 ± 0.6 | 105.9 ± 4.6 | 1.3 ± 0.1 | 14.4 ± 4.3 | 1.5 ± 0.5 |
| | 40% Albumin (1:1) | 136.2 ± 3.5 | 4.6 ± 0.6 | 104.3 ± 3.1 | 1.3 ± 0.0 | 14.1 ± 2.5 | 1.5 ± 0.5 |
| | 40% Starch (1:1) | 136.4 ± 2.8 | 4.9 ± 0.3 | 104.2 ± 3.0 | 1.2 ± 0.1 | 17.4 ± 3.5 | 1.7 ± 0.6 |
| | 30% Starch (1:1) | 136.8 ± 1.7 | 5.2 ± 0.8 | 104.2 ± 2.7 | 1.3 ± 0.1 | 16.0 ± 4.7 | 1.8 ± 0.5 |
| 30 Min. | Control | 139.3 ± 3.1 | 4.6 ± 0.6 | 109.4 ± 4.9 | 1.2 ± 0.1 | 10.4 ± 1.4 | 1.7 ± 0.5 |
| | 40% Saline (3:1) | 143.7 ± 9.1*$ | 4.4 ± 0.5 | 117.9 ± 9.4*# | 1.3 ± 0.1 | 10.6 ± 2.3 | 1.5 ± 0.4 |
| | 40% Albumin (1:1) | 140.6 ± 1.7* | 4.4 ± 0.4 | 108.4 ± 2.3* | 1.3 ± 0.1 | 9.9 ± 1.1 | 1.9 ± 0.4 |
| | 40% Starch (1:1) | 139.4 ± 3.0* | 4.5 ± 0.5 | 111.5 ± 2.6* | 1.2 ± 0.1 | 12.7 ± 5.1 | 2.0 ± 0.3 |
| | 30% Starch (1:1) | 139.6 ± 2.1* | 4.2 ± 0.4 | 111.1 ± 2.8* | 1.2 ± 0.2 | 10.6 ± 2.0 | 2.1 ± 0.5 |
| 60 Min. | Control | 139.0 ± 4.0 | 4.2 ± 0.5 | 110.6 ± 4.9 | 1.2 ± 0.2 | 9.4 ± 1.7 | 1.7 ± 0.5 |
| | 40% Saline (3:1) | 143.4 ± 6.4 | 4.2 ± 0.3 | 117.9 ± 8.7 | 1.3 ± 0.2 | 9.9 ± 1.3 | 1.6 ± 0.2 |
| | 40% Albumin (1:1) | 140.8 ± 1.5 | 4.3 ± 0.4 | 108.6 ± 2.2 | 1.3 ± 0.1 | 9.6 ± 1.4 | 1.9 ± 0.4 |
| | 40% Starch (1:1) | 139.2 ± 3.7 | 4.5 ± 0.6 | 111.8 ± 3.1 | 1.3 ± 0.1 | 13.3 ± 5.6 | 1.9 ± 0.5 |
| | 30% Starch (1:1) | 140.1 ± 1.8 | 4.1 ± 0.2 | 111.3 ± 2.5 | 1.3 ± 0.1 | 10.3 ± 1.3 | 1.8 ± 0.5 |

*; $p<0.05$ vs Baseline. $; $p<0.05$ vs Control. #; $p<0.05$ vs all other groups.

Table 3. Echocardiogram data.

| | Hemodilution | Heart Rate (bpm) | Diastole Volume (μL) | Systole Volume (μL) | Ejection Fraction (%) | Cardiac Output (mL/min) |
|---|---|---|---|---|---|---|
| Baseline | Control | 336 ± 30 | 294.2 ± 94.4 | 71.5 ± 55.5 | 77.1 ± 14.0 | 75.8 ± 30.6 |
| | 40% Saline (3:1) | 376 ± 40 | 314.8 ± 64.3 | 70.6 ± 25.5 | 77.8 ± 5.4 | 91.3 ± 19.0 |
| | 40% Albumin (1:1) | 375 ± 63 | 322.8 ± 40.8 | 84.5 ± 26.0 | 74.0 ± 6.5 | 89.3 ± 19.3 |
| | 40% Starch (1:1) | 353 ± 31 | 331.5 ± 47.3 | 70.0 ± 26.9 | 78.8 ± 7.2 | 92.1 ± 16.9 |
| | 30% Starch (1:1) | 393 ± 31 | 323.4 ± 52.4 | 94.5 ± 32.7 | 71.6 ± 6.7 | 89.9 ± 11.9 |
| 30 Minutes | Control | 326 ± 39 | 331.8 ± 57.7 | 59.0 ± 30.9 | 82.8 ± 7.4 | 89.4 ± 19.1 |
| | 40% Saline (3:1) | 308 ± 24 | 282.6 ± 44.8 | 66.9 ± 16.9 | 76.3 ± 4.6 | 66.2 ± 10.9* |
| | 40% Albumin (1:1) | 361 ± 31 | 359.3 ± 37.6 | 58.7 ± 9.9 | 83.7 ± 2.2 | 108.4 ± 13.2*$ |
| | 40% Starch (1:1) | 332 ± 26 | 346.7 ± 81.4 | 52.9 ± 26.8 | 84.6 ± 6.6 | 97.2 ± 26.4 |
| | 30% Starch (1:1) | 330 ± 28 | 316.9 ± 47.0 | 70.1 ± 26.0 | 78.2 ± 6.3 | 81.7 ± 15.8 |
| 60 Minutes | Control | 315 ± 45 | 347.4 ± 51.2 | 73.8 ± 25.3 | 78.8 ± 6.9 | 86.6 ± 22.2 |
| | 40% Saline (3:1) | 336 ± 29 | 305.4 ± 25.9 | 55.3 ± 17.2 | 82.1 ± 4.7 | 83.7 ± 5.5 |
| | 40% Albumin (1:1) | 354 ± 35 | 352.2 ± 70.5 | 61.8 ± 26.4 | 82.8 ± 6.9 | 102.4 ± 19.8 |
| | 40% Starch (1:1) | 321 ± 42 | 327.3 ± 63.3 | 50.7 ± 38.8 | 85.4 ± 9.3 | 88.3 ± 15.7 |
| | 30% Starch (1:1) | 347 ± 46 | 308.6 ± 45.2 | 69.0 ± 35.4 | 78.4 ± 9.4 | 83.4 ± 15.4 |

Table 4. Primers for qtPCR

| Primers | Sequence |
|---|---|
| Rat RPL13a F | 5’-ATGAACACGCAACCCGTCTC-3’ |
| Rat RPL13a R | 5’-CACCATCCGCTTTTTCTTGT-3’ |
| Rat GAPDH F | 5’-GGATGCAGGGATGATGTTCT-3’ |
| Rat GAPDH R | 5’-GAAGGGCTCATTGACCACAGTT-3’ |
| Rat Glut 1 F | 5’-CACGATACTCAGATAGGACATCC-3’ |
| Rat Glut 1 R | 5’-ACTGTGGTGTCGCTGTTC-3’ |
| Rat Epo F | 5’-GCCTGTTCTTCCACCTTCA-3’ |
| Rat Epo R | 5’-GGAGGCAGAAAATGTCACAATG-3’ |
| Rat VEGF F | 5’-CCCTGGCTTTACTGCTGTACCT-3’ |
| Rat VEGF R | 5’-TCCATGAACTTCACCACTTGATG-3’ |