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Original Articles

Role of Cytokine Hemoadsorption in Cardiopulmonary Bypass-Induced Ventricular Dysfunction in a Porcine Model

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Abstract: Little is known about the effect of cardiopulmonary bypass alone on cardiac function; in an attempt to illuminate this relationship and test a possible mechanism, we used Cytosorb™, a device capable of removing virtually all types of circulating cytokines to test the hypothesis that hemoadsorption of cytokines during bypass attenuates bypass-induced acute organ dysfunction. Twelve Yorkshire pigs (50–65 kg) were instrumented with a left ventricular conductance catheter. Baseline mechanics and cytokine expression (tumor necrosis factor [TNF], interleukin-6 [IL-6], and interleukin-10) were measured before and hourly after 1 hour of normothermic cardiopulmonary bypass. Animals underwent bypass without (cardiopulmonary bypass [CPB], n = 6) or with (CPB+HA, n = 6) the Cytosorb™ device. Data were compared with "historical" controls (n = 6) that were similarly instrumented but underwent observation instead of bypass. Five hours after separation from bypass (or observation), animals were euthanized. Myocardial water content was determined postmortem. Neither TNF nor IL-6 was significantly elevated in either experimental group versus controls at any time point. Preload recruitable stroke work and dP/dt_{max} were significantly depressed immediately after separation from bypass in both CPB+HA and CPB and remained depressed for the duration of the experiment. Although Tau remained unchanged, dP/dT_{min} was significantly diminished in both bypass groups at all time points after separation from bypass. Cytokine hemoadsorption had no effect on any measurable index of function. Differences in postmortem data were not evident between groups. One hour of normothermic CPB results in a significant and sustained decline in left ventricular function that appears unrelated to changes in cytokine expression. Because we did not appreciate a significant change in cytokine concentrations postbypass, the capacity of cytokine hemoadsorption to attenuate CPB-induced ventricular dysfunction could not be assessed. **Keywords:** cardiopulmonary bypass, CPB, LV function, cytokines, filter. *JECT. 2013;45:220–227*

Although it has long been established that cardiac surgery routinely provokes a diffuse systemic inflammatory reaction mediated by complement, fibrinolytic, cytokine, and kinnogen/bradykinin pathways (1,2) and that this reaction has the potential to affect virtually every organ system, little is known about the isolated effects of cardiopulmonary bypass (CPB) alone. This is partially explained by the fact that although extracorporeal circulation is an important contributor to systemic inflammation, other aspects of cardiac surgery contribute to the humoral and cellular response including cardiotomy suction, ischemia–reperfusion injury, surgical trauma, and protamine administration, thus confounding clinical attempts to isolate the effects of CPB. In fact, although it is almost an article of faith that "CPB" is responsible for morbidity associated with surgery, almost all of the studies that describe this "postperfusion" effect use the phrase "CPB" as a surrogate for the many aspects of cardiac surgery that contribute to systemic inflammation. To our knowledge, only one study has been published that addresses the isolated effect of CPB on ventricular mechanics; it demonstrated a

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Dr. John Kellum is on the faculty at the University of Pittsburgh, which holds intellectual property that has been licensed to Cytosobents Inc. for sepsis; he is also a consultant for the company.
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significant decline in both left ventricular contractility and compliance 15 minutes after separation from CPB in a porcine model but did not examine the duration of this effect or potentially responsible mechanisms (3).

Although the systemic response to cardiac surgery is clearly multifaceted, accumulating evidence suggests that surges in inflammatory cytokines may play an important role in postoperative ventricular dysfunction. For example, exogenous cytokine administration has been shown to cause a dose-dependent, reversible injury to ventricular function both in clinical (4) and experimental settings (5). In addition, a correlation between increased tumor necrosis factor (TNF) and interleukin (IL) 6 expression and dysfunctional human donor hearts has been demonstrated (6) and perhaps most relevantly, an association has been demonstrated between proinflammatory cytokines and myocardial dysfunction after cardiac surgery (7). These studies focused our interest on the potential relationship between the effects of CPB on ventricular function and changes in circulating inflammatory cytokine levels.

Although it would have been impractical to attempt measurement of all known inflammatory cytokines, the development of a novel cytokine hemoadsorption filter, Cytosorb™ (Cytosorbents Inc, Monmouth Junction, NJ), provides the opportunity to remove virtually all known cytokines from the bloodstream during the period of CPB by incorporating the hemoadsorption filter into the extracorporeal perfusion apparatus and thus is ideally suited to examine the impact of circulating cytokines on the sequelae of CPB. The Cytosorb™ filter has been shown to significantly reduce cytokine levels both in vitro (8) and in animals with sepsis (9) and the feasibility of hemoadsorption in the organ donor population has already been demonstrated (10). In addition, previous work from our laboratory has demonstrated the use of cytokine hemoadsorption in attenuating brain death-induced ventricular dysfunction in an open-chest porcine model (11). Therefore, we designed this experiment to isolate the effect of CPB on ventricular mechanics and explore the role of inflammatory cytokines by testing the hypothesis that use of a bypass circuit containing a cytokine hemoadsorption filter would attenuate CPB-induced acute organ injury.

MATERIALS AND METHODS

All experiments were approved by the University of Washington Institution for Animal Care and Use Committee and were in compliance with the *Principles of Laboratory Animal Care* formulated by the Institute of Laboratory Animal Resources and with the seventh update of the *Guide for the Care and Use of Laboratory Animals* prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996, www.nap.edu/catalog/5140.html).

Experimental Model

In highly inbred, domestic, male, Yorkshire pigs (n = 18) (range 50–65 kg), ventricular function and cytokine expression in addition to laboratory indices of pulmonary, renal, and hepatic function were measured at baseline and at hourly intervals, for 5 hours, after a 1-hour CPB run using a conventional circuit (CPB, n = 6) or one that incorporated the Cytosorb™ hemoadsorption filter (CPB+HA, n = 6). A third group of animals (historical controls, n = 6) did not undergo CPB. [The current experiment was conducted simultaneously with another experiment from our laboratory [11]. The two experiments were designed to have the same control group to enhance efficiency and altruism. In acknowledgment that the data from the other experiment, including this control group, have already been accepted for publication, for the purpose of the present study, this group is referred to as "historical controls" despite the contemporaneous acquisition of data.) Five hours after separation from CPB (or observation), all animals' hearts were arrested using standard crystalloid cardioplegia after bilateral venting and aortic cross-clamping. Myocardial water content was determined postmortem (Figure 1).

Surgical Preparation

Animals were premedicated using intramuscular telazol (2.0–8.0 mg/kg) and xylazine (2.2–4.4 mg/kg). They were induced with 3–5% isoflurane by facemask, endotracheally intubated, maintained on 1–2% isoflurane and mechanically ventilated at an initial minute volume of 150 mL/kg. Adjustments in ventilation parameters were made based on arterial blood gases that were sent every 15 minutes to maintain a pH of 7.35–7.45 and a carbon dioxide tension 35–45 torr; the fraction of inspired oxygen was kept at 1.0 throughout the experiment. Normothermia was maintained using a Bair Hugger. During the experiments, normal saline was administered through an 18-gauge angiocatheter in an ear vein at 30 mL/kg/h for the first hour and 10 mL/kg/h for the duration of the study. Continuous monitoring included heart rate, body temperature, respiratory rate, electrocardiogram, and mean arterial pressure (through the femoral artery). A median sternotomy was performed and the inferior vena cava was dissected free and encircled with an umbilical tape. A 7-Fr pressure-volume conductance catheter (SciSense, London, Ontario, Canada) was placed through a 4-0 Prolene pursestring suture in the left ventricular (LV) apex.

CARDIOPULMONARY BYPASS CIRCUIT

In the two groups that underwent CPB, animals were given 300 IU/kg of heparin intravenously and placed on

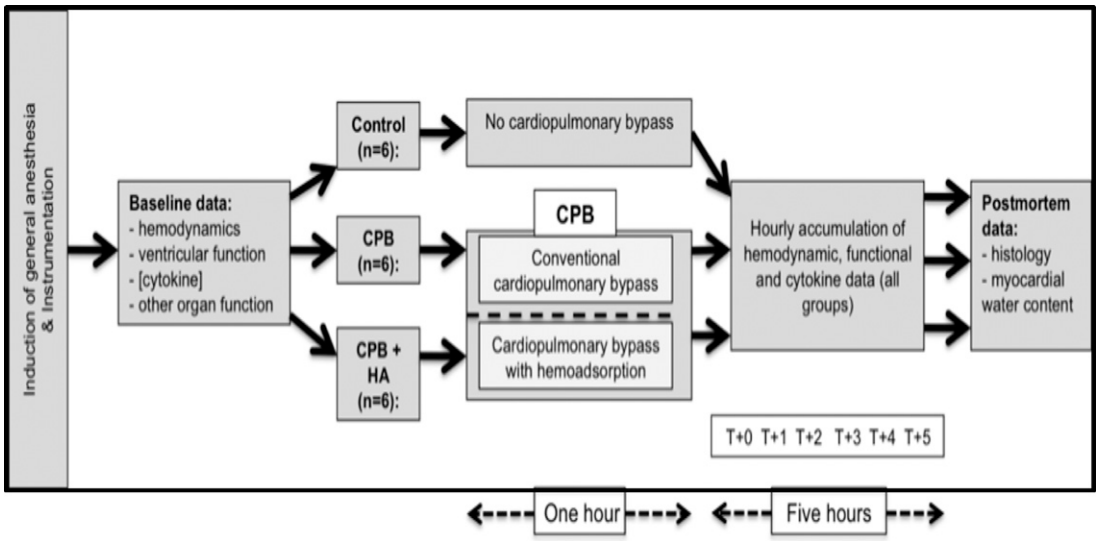

Figure 1. Schematic of experimental design.

bypass using a standard two-staged venous cannula introduced through the right atrial appendage and a 6-mm flow dispersion cannula placed in the ascending aorta. Both cannulae were deaired and attached to the appropriate limb of the tip-to-tip biocompatible perfusion circuit that included a roller pump (Stöckert, Freiburg im Breisgau, Germany), a membrane oxygenator (Medtronic Affinity, Minneapolis, MN), and a closed venous reservoir bag (Medtronic, Minneapolis, MN). The circuit was primed with 600 mL of Plasmalyte-A (Baxter, Deerfield, IL). Normothermic CPB was established with flows 50 mL/kg/min. After full flow was established, the ventilator was turned off. Pressures, flow, and venous oxygenation were evaluated periodically during the period of CPB and "sighs" were given on the ventilator every 15 minutes. Ventilation was resumed after 50 minutes and 10 minutes later animals were gradually separated from bypass. Residual volume in the perfusion circuit was gradually returned through a large-bore peripheral intravenous line.

Cytokine Hemoadsorption

In the CPB+HA group, an additional circuit was created drawing blood from the outflow of the membrane oxygenator and returning it to the venous reservoir; this circuit contained a 300-mL Cytosorb™ cytokine hemoadsorption filter. The active component of this device consists of adsorbent polymer beads composed of porous polymerized divinylbenzene. The pores are sized to remove molecules less than 50 kD, which includes all known cytokines. A peristaltic pump (MasterFlex; Cole-Parmer, Vernon Hills, IL) was used in conjunction with the circuit. The tubing and filter were primed using an additional 200 mL of Plasmalyte-A. Flow through the circuit was set at approximately 350 mL/min.

Data Analysis

Electrocardiography, heart rate, mean arterial pressure, cardiac output, LV pressure, LV volume, and admittance were sampled at 200 Hz with a 10-channel analog-to-digital converter (IX/228S Data Acquisition System; Iworx, Dover, NH) and recorded on a digital computer (MacBook Pro; Apple Computer, Inc., Cupertino, CA). Instantaneous LV volume was determined by measuring parallel conductance in real-time using the method of Wei (12). Pressure-volume loops were recorded during a transient cessation in ventilation during the steady state and inferior vena caval occlusions. Data were analyzed using commercially available software (LabScribe2; iWorx Systems Inc., Dover, NH). Preload Recruitable Stroke Work (PRSW) was used as a load-independent index of contractility (13). The diastolic relaxation constant Tau is the rate of exponential decay in ventricular pressure during isovolumetric relaxation; it was calculated using the Weiss method (14).

Myocardial Water Content

Full-thickness biopsies were taken from the LV free wall of each heart just after explantation. The biopsy was blotted dry and weighed on a digital scale to determine wet weight (WW); it was then dried in an oven for several days to a constant weight and reweighed to determine dry weight (DW). Myocardial water content (MWC%) was calculated as follows:

$$\mathrm{MWC\%} = [(\mathrm{WW} - \mathrm{DW})/\mathrm{WW}] * 100$$

Biochemical Analyses

Blood samples were drawn into pyrogen-free vials and plasma was separated by centrifugation and frozen (–80°C). Serum concentrations of cytokines previously demonstrated to effect ventricular function (7) (TNF, IL-6, and IL-10)

were measured at baseline and at hourly intervals for the duration of the experiment. Cytokine concentrations were measured using commercially available, swine-specific, enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN) thawing samples only once before using. This assay uses the quantitative sandwich enzyme immunoassay technique. At baseline and at t0 (directly after separation from CPB) and t5 (5 hours after separation), samples were also drawn to measure aspartate aminotransferase, alanine aminotransferase, blood urea nitrogen (BUN), and creatinine and were analyzed by an on-site veterinary laboratory. Plasma neutrophil gelatinase-associated lipocalin (NGAL), a protein expressed in neutrophils and certain epithelia including renal tubules, was used as an early and sensitive biomarker of renal injury using commercially available enzyme-linked immunosorbent assay kits (BioPorto Diagnostics, Gentofte, Denmark).

Statistical Analysis

Serial data over time within a group were analyzed with repeated-measures analysis of variance (ANOVA) and post hoc comparisons were made using the Dunnett test to compare mean values at each time point to baseline values. For comparisons between groups at a given time-point, we used one-way ANOVA with post hoc comparisons made by Bonferroni's method when appropriate. The primary analysis was the determination of significant differences between groups at 5 hours after separation from CPB; we performed the same analysis at other time points but did not adjust for multiple comparisons. When data did not pass the Kolmogorov-Smirnov normality test for Gaussian distribution or if the Bartlett's test suggested that differences in standard deviations were significant, then the nonparametric Kruskal-Wallis test was used with Dunn's post hoc test where appropriate. All p values < .05 were considered statistically significant. Analyses were conducted using GraphPad Prism Version 5 software (GraphPad Software Inc., La Jolla, CA).

Table 1. Hemodynamics and ventricular function over time.

	Baseline	10 Minutes after Separation from CPB	5 Hours after Separation from CPB
Heart rate (bt/min)			
Control	82.1 ± 4.6	79.7 ± 3.7	76.7 ± 3.6
CPB	82.2 ± 6.3	98.0 ± 4.8	94.3 ± 1.7
CPB+HA	76.7 ± 3.6	87.3 ± 9.6	88.8 ± 2.8
Cardiac output (mL/min)			
Control	1732 ± 355	2230 ± 476	**2947 ± 618**[b]
CPB	3839 ± 891	3157 ± 1001	2750 ± 600
CPB+HA	2403 ± 500	2637 ± 333	3654 ± 578
dP/dt_{max} (mmHg/sec)			
Control	787 ± 72	896 ± 65	754 ± 36
CPB	1132 ± 62	**860 ± 40**[b]	**740 ± 66**[b]
CPB+HA	1305 ± 234	**803 ± 93**[b]	**760 ± 68**[b]
dP/dt_{min} (–mmHg/sec)			
Control	1110 ± 64	1180 ± 73	1070 ± 89
CPB	1412 ± 104	**910 ± 68**[b]	**816 ± 86**[b]
CPB+HA	1462 ± 162	**886 ± 94**[b]	**763 ± 44**[a,b]
Ejection fraction (%)			
Control	35 ± 4	42 ± 6	38 ± 4
CPB	51 ± 5	39 ± 4	42 ± 4
CPB+HA	49 ± 8	48 ± 6	52 ± 6
Mean arterial pressure (mmHg)			
Control	60 ± 4	62 ± 5	56 ± 3
CPB	73 ± 5	**53 ± 5**[b]	**45 ± 2**[a,b]
CPB+HA	64 ± 5	**44 ± 3**[a,b]	**40 ± 2**[a,b]

Figures are reported ± standard errors of the mean
[a] $p < .05$ versus control (same time point).
[b] $p < .05$ versus baseline (same group).
CPB, cardiopulmonary bypass; HA, hemoadsorption. Bold represents statistical significance (standard usage).

RESULTS

Overview

There were no significant differences in hemodynamics, indices of ventricular function, or serum markers of renal,

Table 2. Noncardiac organ function over time.

	Baseline	From CPB	10 Minutes after Separation from CPB	5 Hours after Separation from CPB
Creatinine (mg/dL)				
Control		.90 ± .1	1.12 ± .1	1.10 ± .1
CPB		1.12 ± .1	1.25 ± .1	**1.52 ± .1**[a,b]
CPB+HA		1.23 ± .1	1.27 ± .1	**1.42 ± .1**[b]
BUN/creatinine				
Control		7.9 ± .7	7.9 ± .9	10.8 ± .2
CPB		7.1 ± .6	6.1 ± .6	7.9 ± .5
CPB+HA		6.6 ± .7	6.8 ± .6	8.8 ± .8
NGAL (ng/mL)				
Control		146 ± 21	137 ± 20	163 ± 24
CPB		152 ± 16	139 ± 22	150 ± 17
CPB+HA		139 ± 4.1	130 ± 11	135 ± 10
AST (u/L)				
Control		30.3 ± 4.7	35.7 ± 1.5	50.0 ± 7.6
CPB		25.0 ± 2.3	47.8 ± 11	55.4 ± 8.0
CPB+HA		25.2 ± 2.8	35.0 ± 2.7	49.3 ± 7.1
ALT (u/L)				
Control		32.3 ± 5.4	28.7 ± 6.2	28.0 ± 6.0
CPB		30.0 ± 3.6	22.8 ± 2.6	21.6 ± 2.8
CPB+HA		29.5 ± 5.0	20.3 ± 2.8	18.3 ± 2.8
PaO_2/FIO_2				
Control		32.3 ± 5.4	477 ± 37	443 ± 48
CPB		30.0 ± 3.6	**305 ± 66**[b]	449 ± 29
CPB+HA		29.5 ± 5.0	**331 ± 79**[b]	404 ± 32
pH				
Control		7.54 ± .02	7.48 ± .04	7.48 ± .01
CPB		7.51 ± .01	7.41 ± .02	7.50 ± .02
CPB+HA		7.53 ± .02	7.44 ± .04	7.49 ± .01

Figures are reported ± standard errors of the mean.
[a] $p < .05$ versus control (same time point).
[b] $p < .05$ versus baseline (same group).
CPB, cardiopulmonary bypass; HA, hemoadsorption; BUN, blood urea nitrogen; NGAL, neutrophil gelatinase-associated lipocalin; AST, aspartate aminotransferase; ALT, alanine aminotransferase. Bold represents statistical significance (standard).

hepatic, or pulmonary function at baseline (Tables 1 and 2). Similarly, there were no significant differences in any measurable end point between CPB and CPB+HA at any time point. Hemodynamics and indices of both systolic and some indices of diastolic ventricular function were depressed for both experimental groups at virtually all time points after separation from CPB.

Effect of Cardiopulmonary Bypass on Cytokine Concentration and Effectiveness of Cytokine Hemoadsorption

Figure 2A–B demonstrates the effect of CPB on TNF and IL-6 expression and the effect of CytoSorb™ hemoadsorption. CPB did not induce significant serum increases in either measured cytokine when compared with historical controls and there were no differences in measured cytokine concentration between the two groups subjected to CPB. Specifically, in both CPB and CPB +HA, TNF expression did not increase versus baseline within each group; neither was TNF expression significantly greater than historical controls at any time point. For IL-6 expression, again there was no significant difference between groups at any time point; within each group (including historical controls), IL-6 expression was significantly raised at time points 4 and 5.

Effect of Cardiopulmonary Bypass on Systolic Left Ventricular Function

Systolic LV function as reflected by PRSW is demonstrated by Figure 3A. For control animals, PRSW was unchanged for the duration of the experiment. For CPB animals, PRSW was significantly reduced versus baseline at every time point after separation from bypass. It was also significantly reduced versus historical controls for the first 3 hours after separation from bypass. For CPB+HA, PRSW was significantly reduced versus historical controls at t0 and t3 and was significantly reduced versus baseline at t2–t4. Although for CPB, PRSW after separation from bypass was between 74% and 81% of baseline and for CPB+HA it was between 83% and 88%, at no point were these differences statistically significant. LV maximum change in pressure with time (dP/dt_{max}), another measure of systolic function, demonstrated similar trends: it was unchanged over time in historical controls, but for both groups subjected to bypass, it was significantly depressed after separation (Table 1).

Effect of Cardiopulmonary Bypass on Diastolic Left Ventricular Function

Diastolic LV function as reflected by Tau is demonstrated by Figure 3B. There were no significant changes over time for any of the three groups; neither were there significant differences between groups. For LV minimum change in pressure with time (dP/dt_{min}), another index of diastolic function, no changes were evident for historical controls, but for both experimental groups, dP/dt_{min} decreased reflecting diminished ventricular compliance both directly after separation from CPB (t0) and 5 hours later (t5) (Table 1).

Other Hemodynamic Indices

Cardiac output was increased over time versus baseline in historical controls, whereas in CPB and CPB+HA, it was unchanged over time versus baseline and when compared with historical controls (Table 1). Ejection fraction remained unchanged over time in each group, whereas mean arterial pressure (MAP) remained unchanged for historical controls but was significantly diminished versus baseline for both experimental groups. MAP was significantly reduced versus historical controls at t5 for both experimental groups.

Effect of Cardiopulmonary Bypass on Noncardiac Organ Function

Hepatic enzymes, the BUN/creatinine ratio, and blood pH remained unchanged over time for all groups (although changes in ventilation parameters were made to maintain pH within specific parameters) (Table 2). Serum creatinine became significantly elevated for both groups subjected to CPB 5 hours after separation. No animal in either group experienced a doubling of the baseline serum creatinine (RIFLE-I) (15) and NGAL did not increase in any of the groups. Initial blood gases taken directly after separation from CPB (t0) demonstrated a significant decrease in PaO_2/FIO_2 ratio for both CPB and CPB+HA, which normalized at t5.

Postmortem Data

Myocardial water content in historical controls, CPB, and CPB+HA was 74.1 ± .7%, 75.6 ± 1.6%, and 74.7 ± 1.1%, respectively ($p = .26$, ANOVA).

COMMENT

This study demonstrates that 60 minutes of normothermic CPB in a previously healthy animal with normal cardiac function results in a significant and sustained depression of load-independent indices of systolic ventricular function and some indices of diastolic function without meaningful changes in hemodynamics; we did not observe any increase in TNF or IL-6 as a result of the bypass run; neither did we observe any measurable impact of incorporating a cytokine hemoadsorption filter into the conventional CPB circuit suggesting that the observed impact on ventricular function is unlikely related to changes in cytokine concentrations. Statistically significant increases in IL-6 occurred in both groups subjected to CPB as well as the control animals suggesting that this is a response to the trauma of surgical instrumentation rather than an effect related to extracorporeal circulation.

To our knowledge this is the only study directly measuring the impact of CPB alone on cytokine concentrations, although other studies have addressed this relationship obliquely comparing the effect of centrifugal pumps versus roller pumps (16,17), removal of the oxygenator from the CPB circuit (18), and the use of biocompatible circuits (19,20) on cytokine levels after surgery. Our data suggest that increases in inflammatory cytokine concentrations observed clinically are related to aspects of cardiac surgery unrelated to extracorporeal circulation and/or the membrane oxygenator.

Although observed changes in systolic indices were consistent, changes in diastolic indices were not with the load-independent diastolic relaxation constant Tau remaining unchanged, whereas changes in dP/dt_{min} implied diminished ventricular compliance in the groups subjected to CPB. We were somewhat surprised to see significant increases in creatinine within such a short timeframe, and

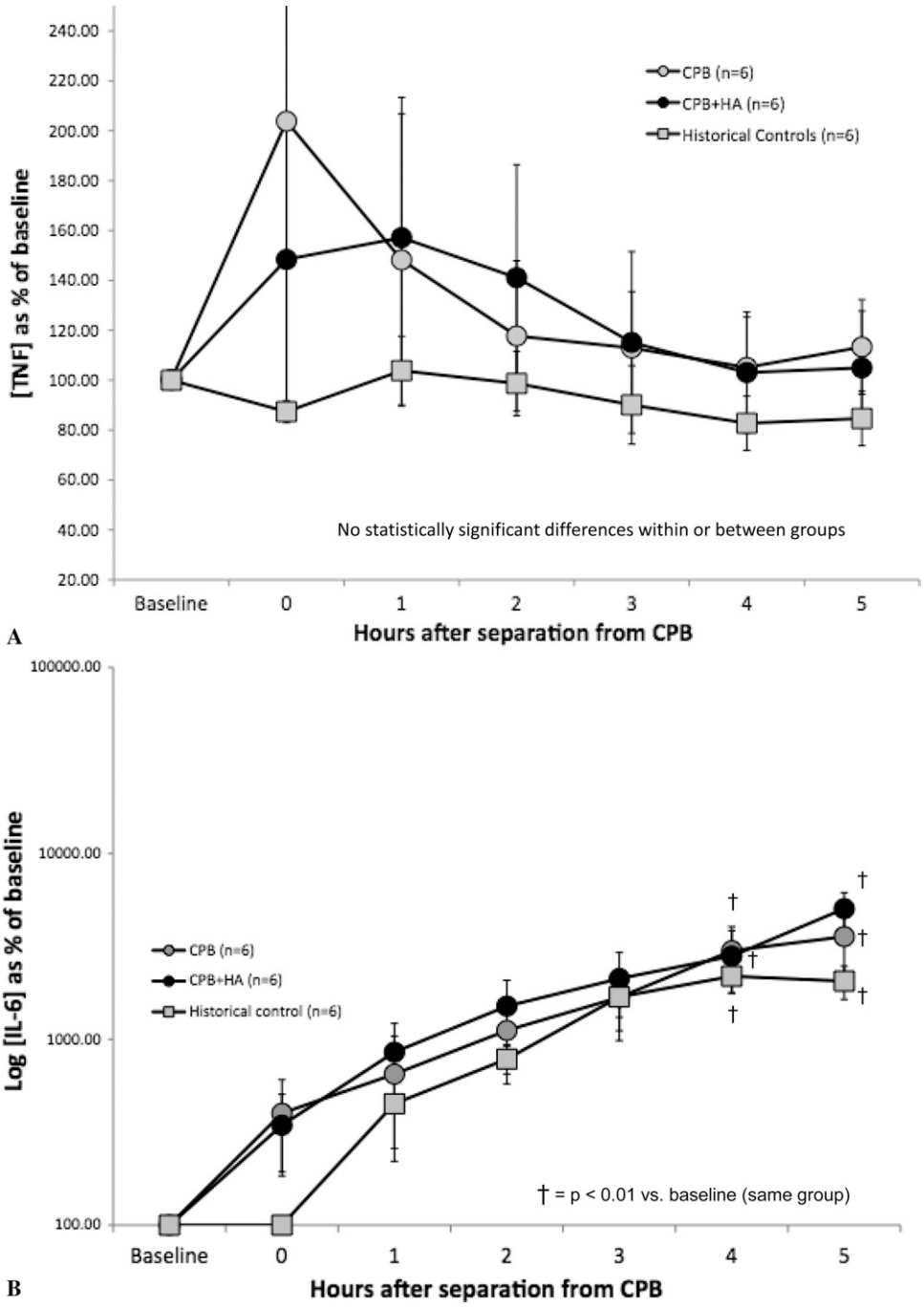

Figure 2. Effect of cytokine hemoadsorption on cardiopulmonary bypass-induced tumor necrosis factor (TNF) expression (A) and interleukin-6 (IL-6) expression (B). Brackets indicate standard errors of the mean.

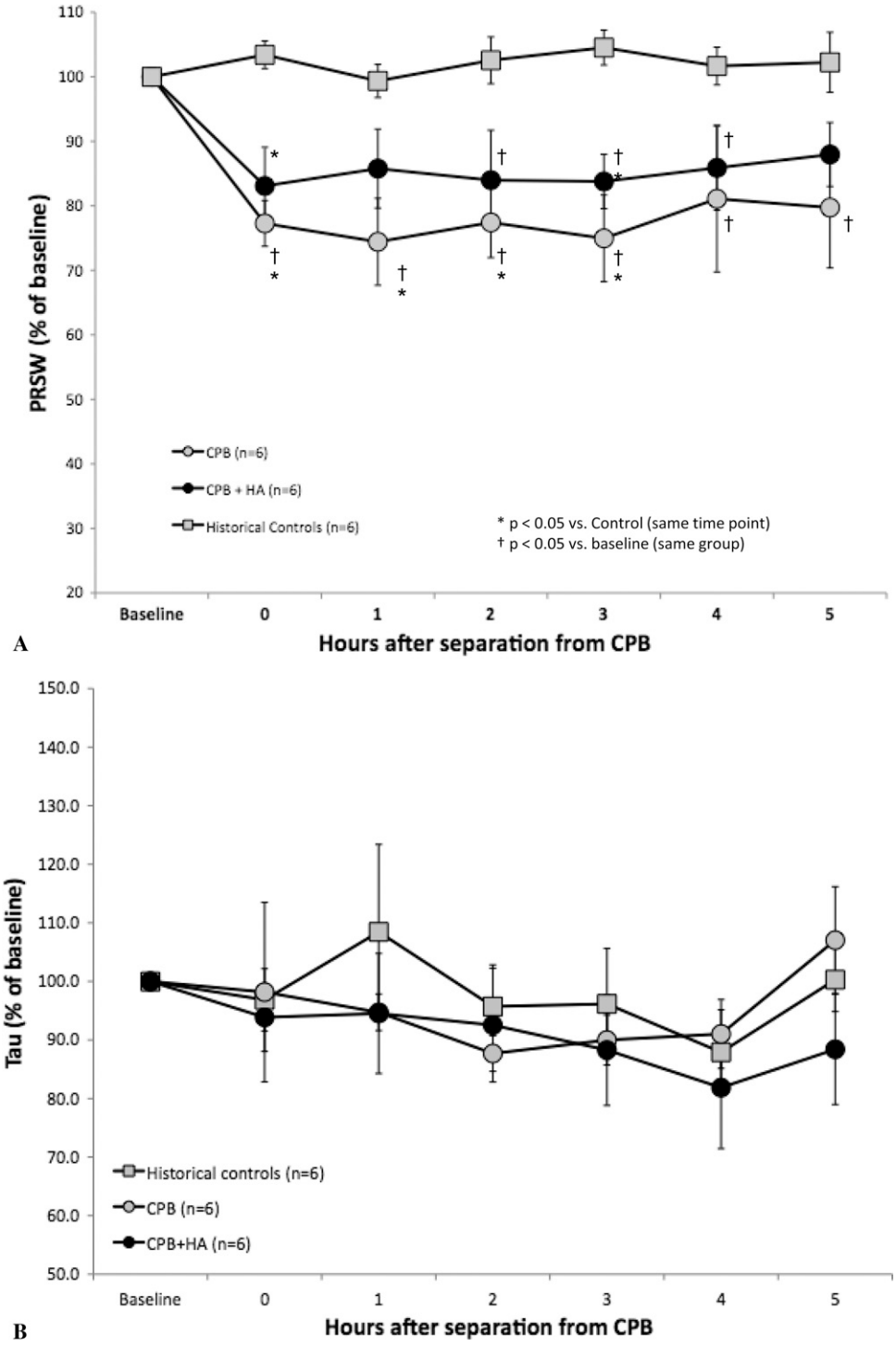

Figure 3. Effect of cytokine hemoadsorption on cardiopulmonary bypass-induced left ventricular systolic (A) and diastolic (B) dysfunction. Brackets indicate standard errors of the mean.

because there were not corresponding changes in NGAL, a more sensitive index of early renal dysfunction, we are reluctant conclude that the bypass run had a significant impact on renal function. Impaired gas exchange directly after separating from CPB might be the result of transient changes in pulmonary function, but the development of atelectatic changes from the apneic period with subsequent shunting may not be excluded.

Explanations for observed changes in ventricular function exclusive of changes in cytokine concentrations include changes in myocardial perfusion caused by nonpulsatile flow (21) hemodilution (22), potential embolic events, or

other components of the inflammatory response. Potentially confounding aspects of our experiment include the possibility that the CytoSorb™ device absorbed molecules in the same size range as cytokines such as catecholamines, cortisol, triiodothyronine, and insulin and thus the possibility that positive inotropic mediators were absorbed along with negative ones giving the appearance of a net-neutral effect; however, given the lack of changes in common inflammatory cytokines that we measured and the lack of significant changes in similar-sized molecules in previous studies of this filter (10), we think this possibility is unlikely. In addition, because it is known that the myocardium is capable of synthesizing biologically active TNF, we cannot exclude the possibility that locally produced agents, unmeasurable by our methods, may have been responsible for the observed myocardial depression (5).

Although our data did not offer evidence of a benefit to incorporating the cytokine hemoadsorption filter into the CPB circuit in this setting, given the lack of observed changes in cytokine concentrations, it would have been surprising if there had been a demonstrable effect. Incorporation of the cytokine filter in a more clinically relevant scenario where surges in inflammatory cytokines have been demonstrated may have a meaningful protective effect. Further investigation into the mechanism by which CPB results in depressed ventricular function is warranted.

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