A RANDOMIZED TRIAL USING A PORCINE MODEL OF DEEP HYPOTHERMIC CIRCULATORY ARREST TO STUDY THE BENEFITS OF BIOLOGICALLY VARIABLE CARDIOPULMONARY BYPASS

BY

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A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
for the Degree of

MASTER OF SCIENCE

Department of Surgery
University of Manitoba
Winnipeg, Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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ABSTRACT

Purpose: Biologically variable perfusion uses a computer-controller to restore physiologic beat-to-beat variability, known to be associated with health, to roller pump flow during cardiopulmonary bypass. We hypothesized it would improve systemic perfusion and decrease renal injury compared to conventional apulsatile perfusion following deep hypothermic circulatory arrest.

Methods: Eighteen pigs were randomized to either biologically variable or apulsatile perfusion as their perfusion strategy. They underwent cardiopulmonary bypass and cooling (1hr), deep hypothermic circulatory arrest at 18°C (1hr) and resumed perfusion with rewarming to normothermia for 3hr. Hemodynamics, acid-base status, body temperature and urine volumes were measured. Urinary markers of tubular injury were compared between groups post-hoc at specified time periods with urine concentrations of gamma glutamyl transpeptidase, alkaline phosphatase and glutathione S-transferase; and by urine proteomics using mass spectrometry.

Results: Total urine output following arrest was 530±216 mL in the biologically variable group compared to 242±75 mL in the apulsatile group ($p<0.002$). Renal injury markers including protein to creatinine ratio, alkaline phosphatase concentration, gamma glutamyl transpeptidase concentration and glutathione s-transferase activity were higher in the apulsatile group at all time points after arrest ($p<0.002$). Urine proteomics verified the presence of abnormal proteins persisting longer in the apulsatile group. Biologically

variable perfusion decreased cooling time to 21.0±9.0 min compared to 31.7±7.5 min, and decreased rewarming to 22.1±3.9 min versus 31.2±5.1 min ($p<0.002$).

Conclusions: Perfusion was superior with biologically variable perfusion as suggested by improved urine output, decreased markers of tubular injury, attenuated mass spectrometry urine protein signal and more rapid temperature changes. This strategy could potentially shorten bypass duration and decrease renal injury with deep hypothermic circulatory arrest.

INTRODUCTION

Cardiopulmonary bypass (CPB) has been the cornerstone of cardiac surgery for the past 50 years[1]. This technology evolved from advances in roller pump design ongoing from 1855[2]. CPB is the approach to extracorporeal oxygenation and perfusion developed initially by Gibbon in 1953, and subsequently brought to the clinical arena by Lillehei at the University of Minnesota and by Kirklin at the Mayo Clinic[3]. This incredible development has allowed surgery to take place on an arrested heart with correction of complicated intracardiac lesions while maintaining the patient's viability through extracorporeal support. Optimizing strategies with respect to flow characteristics, myocardial protection, fluid composition and a change from the original bubble oxygenator developed by Dewall[3] to the currently accepted membrane oxygenator have improved efficiency and decreased complications, but the basic strategy of perfusion has remained largely constant. Nevertheless, the utility of CPB in cardiac surgery has led to increasing use such that the peak number of cases in the United States alone exceeded 450,000 cases in 1999[3].

CPB is not without its detriments and complications. Extracorporeal circulation is associated with hypotension, hemodilution, altered circulating catecholamines, complement activation and a generalized inflammatory response[4]. Importantly, the lack of physiologic flow with conventional apulsatile CPB has been implicated in end organ dysfunction[5]. Two of the main organ systems affected are the neurologic and renal systems, however all end organs suffer some degree of disruption as a result of CPB. Transient neurologic dysfunction occurs in up to 60% of cases with a rate of cerebrovascular accident of 2% - 5%[6]. Shaw et al. found moderate or severe intellectual

dysfunction occurred in 24% of 312 CPB patients compared to 0% in 50 reasonably matched control patients undergoing peripheral vascular surgery[7]. Gastrointestinal complications occur in 0.6% - 2.0% of cardiac operations with a subsequent mortality of 15% - 63%[4]. Hepatic blood flow has been demonstrated to decrease with an incidence of post-CPB hyperbilirubinemia of approximately 20%[8].

One of the outcomes of interest addressed in this thesis is the complication of acute renal failure (ARF). It is well known that ARF has a negative impact on survival for all hospitalized patients. Shusterman et al. quantified the relative risk of mortality at 6.2 with a 95% confidence interval of 2.6 - 14.9 in a case-controlled study of 762 eligible patients[9]. Mortality with ARF has been prospectively found to be 64% in 2,216 consecutively studied medical and surgical patients[10].

With respect to the cardiac surgical setting, significant renal dysfunction associated with cardiac surgery and duration of cardiopulmonary bypass have been documented since the 1970's[11,12]. In recent times, two extensive studies have been conducted to characterize the incidence and impact of ARF associated with cardiac surgery as well as describing associated covariates. The first was published in 1994 by Chertow et al. in which a prospective cohort study of 43,642 patients undergoing coronary artery bypass or valve surgery between 1987 and 1994 were studied[13]. The overall risk of ARF requiring dialysis was 1.1% with a subsequent mortality of 63.7%. Notable independent risk factors for this complication were valvular surgery, poor baseline renal function, New York Heart Association (NYHA) class IV heart failure, preoperative need for an intra-aortic balloon pump (IABP), ejection fraction (EF) less than 35%, prior heart surgery and peripheral vascular disease. The overall risk of ARF

was not presented, however, it was characterized as being greater than 5% in the highest risk groups. Intraoperative variables such as duration of CPB were not examined.

The second major multicenter examination was published in 1998 by Mangano et al.; a prospective, observational study of 2,222 patients having myocardial revascularization[14]. This showed an overall rate of ARF of 7.7% with a mortality of 19%, intensive care unit (ICU) stay of 6.8 days and hospital stay of 18.2 days. When dialysis was required (1.4% overall) outcomes worsened with mortality of 63%, ICU stay of 14.9 days and hospital stay of 28.8 days. These were in stark contrast to patients with normal renal function with post op mortality of 0.9%, 3.1 ICU days and 10.6 hospital days respectively. Furthermore patients with renal dysfunction were 3 times as likely to be discharged to a long term care facility. The multivariable analysis demonstrated that duration of CPB lasting greater than 3 hours was an independent risk factor for ARF with a relative risk of 2.8 and a 95% confidence interval of 1.6 – 4.9.

More recent studies have attempted to confirm the association between CPB, ARF and poor outcomes. Conlon et al. published data in 1999 where they identified duration of CPB as an independent risk factor by multivariable analysis[15]. In their study, 7.3% of 2,843 consecutive patients undergoing cardiac surgery with CPB had renal failure with a mortality of 14%. Mortality was 28% when dialysis was required. Boldt et al. compared patients with CPB duration of <50 minutes to patients with times of >90 minutes and found significantly greater renal injury in the latter group[16].

Finally, studies which attempt to compare reasonably similar patients undergoing CPB versus revascularization strategies that do not require CPB have found higher rates of ARF[17] and increased markers of renal damage[18,19] in patients where CPB was used.

These and other detailed examinations of renal failure in cardiac surgery provide compelling evidence that CPB is associated with ARF; with an incidence of 1% - 30% and a subsequent mortality of 7% - 38%[11-19]. Furthermore, these mortality rates increase tremendously when dialysis is needed. There is little doubt that optimizing CPB to reduce renal and other complications is an important endeavour. Strategies to do this include: i) eliminating the technique altogether by 'off-pump' approaches or ii) attempts at 'physiologic' modifications by adding pulsation. A recent study examining perfusion practice in Canada indicates that off-pump surgery takes place in the minority of cases in most centers[20].

Pulsatile CPB has been explored since the 1970's in many different ways with mixed results[21]. For example, benefits on vital organ function were suggested with use of an IABP to generate pulsatility during CPB in 1975[22]. Since then there has been a broad range of outcomes which have demonstrated potentially better organ flows with the addition of pulsatility, but mixed results with respect to brain[23] and renal protection [24], and stress hormone[25] and activated complement release[26,27]. While advocates of pulsatile CPB recommend its use during moderate- to high-risk cardiac surgery[28], this has not become the standard practice as a result of non-standardized definitions of pulsatility, poorly conducted trials and inconsistent results. The benefit of pulsatile perfusion is still highly controversial[29].

Pulsatile models attempt to make CPB more physiologic by incorporating a pulse pressure typical of normal circulation as opposed to the rectified sine wave seen with conventional apulsatile roller pump CPB[30,31]. Although pulsation is achieved in a variety of ways with numerous different mechanical strategies for doing so, a consistent feature

of these systems is the monotonous nature of the pulsation[22-31]. There is no incorporation of beat-to-beat variability as is seen in normal physiology. The lack of biologic variability in the currently investigated monotonous pulsation (MP) may be of critical importance in its failure to demonstrate a consistent benefit over conventional, apulsatile continuous perfusion (CP).

The Concept of Variability in Normal Health: Recent investigation into the importance of biologic variability is increasingly bringing forth evidence that this variability is of physiologic utility. Buchman's review article, 'The Community of the Self', summarizes the recognition of the importance of the dynamic, nonlinear functioning of the various body systems in concert with one another[32]. Whereas traditionally, medical practice takes a reductionist approach that uses mean values and attempts to restore functioning to defined homeostatic norms, Buchman states how new insights, combined with the addition of increasing computational capability, show that filtering out variations and fluctuations by such practice may be ignoring important features of physiologic functioning. Normalization of blood pressure in penetrating trauma, infusion of calcium to correct hypocalcemia of sepsis and hormone therapies to replace menopausal deficiencies are cited examples where attempting to maintain the normalized status-quo have proved to be harmful. In fact, it is now felt that one of the most important properties of complex nonlinear systems is their paradoxical ability to generate negative entropy and more order[33]. Thus instituting therapies which do not account for nonlinear functioning may be detrimental.

Ongoing investigation has characterized the nature of the variability in many healthy physiologic processes as being fractal in nature. Heart rate, respiratory rate and blood pressure all are fractal in their variability characteristics[34,35]. There is utility in such variation. For example, respiratory sinus arrhythmia, a phenomenon which has been known to exist for over a century, is now being shown to be associated with health and to improve gas exchange and circulatory efficiency [36-38]. Furthermore, the presence or absence of healthy variation can be used to prognosticate patient outcomes[39]. The presence and coordination of these variable processes are felt to be important because they: i) serve as a self organizing mechanism for highly complex processes and ii) allow for a wider functional response compared to highly periodic behaviours[40,41]. With respect to this it has been demonstrated that the variability of such processes is lost in non-healthy states. By way of example the heart rate in heart failure[42,43] and gait in people with Parkinson's disease both lose their characteristic fractal variability[40]. Finally, in the critical care setting, there is evidence that the pathogenesis of multiple organ dysfunction syndrome (MODS) is a consequence of physiologic uncoupling of vital organ systems[44,45]. Consequently we are now appreciating that biologic variability is a feature of health that serves a complex physiologic function and that modern technology allows us not only to interpret, but to mimic it as well. Standard life support systems (ie. mechanical ventilators, apulsatile CPB, monotonously pulsatile CPB) result in a loss of normal fractal transmission and therefore, connection between organ systems[46]. This may represent an iatrogenic, physiologic uncoupling akin to that seen in MODS.

Experimental investigation of different life support systems has shown that reintroduction of biologic variability with computer controlled interventions is beneficial.

Adding biologic variability to a mechanical ventilator improves oxygenation and respiratory mechanics compared to monotonous delivery of fixed volumes and rates with a conventional ventilator[47-51]. More relevant to this thesis, using a computer controller to introduce biologic (fractal) variability to the CPB roller pump resulted in improved jugular venous oxygen saturation ($SjvO_2$) in pigs during rewarming compared to CP or MP[52,53]. As $SjvO_2$ less than 50% is correlated with poor neurocognitive outcomes[54] the area under the curve for $SjvO_2$ below 50% for each technique was calculated. The ratio for the area below 50% saturation from biologically variable CPB (BVP) to MP to CP was 1 to 20 to 75, indicating superior oxygenation with BVP compared to either monotonously pulsatile or apulsatile perfusion, suggesting BVP was a better perfusion strategy. Similar conclusions were found when comparing biologically variable delivery of cardioplegia to continuous flow, in which diastolic performance was preserved at normal levels in the variable group compared to a decrease in diastolic compliance with continuous flow cardioplegia[55]. Plus, evidence in support of the use of biologic variability in life support systems is growing in a variety of applications.

In summary, CPB is a tremendous advance but it is not without its complications such as neurocognitive dysfunction and renal failure. Renal failure post CPB carries an extremely poor prognosis. Attempts to reduce these complications by modifying CPB using pulsatile techniques are controversial at this stage. However, the inability of current techniques to improve outcomes may be related to their lack of biologic variability which is increasingly shown to be associated with health; intrinsic in many body systems which are coupled together and which degrades with age and in disease. Previous

experimentation has demonstrated a benefit of incorporation of biologic variability to life support systems. Thus, BVP may enhance perfusion and diminish complications of CPB.

In order to examine the benefits of BVP on systemic perfusion and consequent renal integrity and function, we designed a porcine model of CPB in which deep hypothermic circulatory arrest (DHCA) was used to initiate a renal insult, followed by 3 hrs of reperfusion with either BVP or CP. We compared groups in terms of indices of systemic perfusion and renal injury. The degree of renal injury was characterized by protein markers in urine, urine proteomics and histological analysis. The urinary protein markers included glutathione S-transferase (GST), gamma glutamyl transpeptidase (GGT) and alkaline phosphatase (AP). The former is a cytosolic enzyme while the latter two are brush border enzymes, seen principally in the proximal convoluted tubular cells. They appear in the urine as they leak into the collecting system as a consequence of renal tubular damage analogous to troponin release seen cardiac myocyte damage[56]. They have all been validated as sensitive measures of renal insult at an earlier time than traditional measures of renal function using serum creatinine, urine output or creatinine clearance[56,57]. Our hypothesis was that perfusion would be improved with BVP and would result in decreased renal injury compared to CP.

MATERIALS AND METHODS

A series of pilot studies were conducted in order to establish the final protocol which would most appropriately assess the hypothesis. All studies underwent some variation of a similar strategy of anesthesia (at a minimum) and further endeavours in CPB and DHCA.

Certain features were common to all experiments including the final protocol. All experiments were performed on female pigs weighing 30-40 kg and all animals were treated according to the guidelines of the Canadian Council on Animal Care as approved by the Committee for Animal Experimentation at the University of Manitoba, Winnipeg, Canada.

In all experiments, the pigs were sedated with intramuscular ketamine 12 mg/kg, atropine 0.6 mg/kg and midazolam 0.6 mg/kg followed by 4% isoflurane in oxygen by nose cone prior to endotracheal intubation and mechanical ventilation. Anesthesia was maintained with isoflurane 2% in oxygen. Pancuronium bromide was given by continuous intravenous infusion of 10 mg/hr for muscle relaxation. The bladder was catheterized. The right femoral artery and external jugular vein were cannulated. Nasopharyngeal temperature probes were inserted. Central venous pressure (CVP), mean arterial blood pressure (MAP), arterial blood gases, hemoglobin, serum and urinary electrolytes, liver enzymes and lactate, urine output and temperature were monitored throughout. All hemodynamic data were recorded continuously on a Gould 2600 oscillograph (Gould, Cleveland, OH) and to an analog to digital data acquisition system (Advanced Codas, Dataq Instruments, Akron, OH). Arterial blood gases were analyzed using a Radiometer ABL 500 (Copenhagen, NV, Denmark) and hemoglobin and mixed

venous oxygen saturation was measured using a Radiometer OSM3 set for porcine blood. Three animals underwent no further intervention than the anesthetic apart from systemic heparinization as conducted in all animals and described later (CTL Group).

The other pigs all underwent a similar approach to cannulation and CPB. A midline sternotomy was performed and the aorta and right atrium cannulated (6.5 mm; Stockert GmbH, Freiburg, Germany and 32F DLP single-stage cannula; DLP, Inc, Walker, MI respectively) to initiate CPB following administration of 20,000 units of intravenous heparin. Baseline blood and urine samples were collected. CPB was initiated with a prime of 1L of lactated Ringer's solution, 500 mL of Pentaspan and 5,000 IU of heparin. Extracorporeal oxygenation was maintained by membrane oxygenator (Cobe Optima 4700). Alpha-stat pH management and an in-line arterial filter (Medtronic Affinity 38μm) were used. Heparinization was maintained with 5,000 IU intravenously every hour. Once stable on CPB, mechanical ventilation was terminated and potassium chloride, 40 mEq, was injected into the left ventricle. A cross-clamp was applied to the ascending aorta, proximal to the arterial CPB cannula. At all times during CPB, 200 mL of lactated Ringer's was added if the volume in the venous reservoir fell below 200 mL; these volumes were recorded. The various pilot studies and the final protocol used differing doses of mannitol and varied durations and temperatures of CPB and circulatory arrest (CA) which will be elaborated individually for each group below.

At end experiment, the animal was sacrificed and the right kidney was harvested following laparotomy. The renal artery was cannulated and the kidney was perfused with iced saline until the venous effluent appeared clear. Sections of the kidney were removed and snap frozen in liquid nitrogen and the remaining tissue was perfused with 1L of 5%

formalin for perfusion-fixation. Blood and urine samples were collected at set intervals for each protocol as discussed below.

Glutathione S-transferase (GST) measurement: The activity of urinary glutathione S-transferase in urine was measured using a spectrophotometric enzymatic assay for total GST activity (both cytosolic and microsomal). (Cayman Chemical Company, Ann Arbor, MI). All procedures were performed according to the kit instructions; with changes in absorbance levels at 340nm measured every two minutes for a total of twelve minutes. The increase in absorbance was plotted against time and the resulting slope was converted into enzyme activity in units of nmol/min/mL.

Gamma glutamyl transpeptidase (GGT) measurement and Alkaline Phosphatase (AP): GGT, AP and protein to creatinine ratios were quantified using reflectance spectrophotometry with a Vitros 700 analyzer (Ortho-Clinical Diagnostics, Inc., Rochester, NY), performed at the University of Manitoba's Veterinary Laboratory.

Histology: The formalin-fixed, paraffin embedded kidney tissue blocks were stained with periodic acid Schiff (PAS) stain. The stained histologic sections were assessed in a blinded fashion by an experienced nephropathologist. To investigate the possibility of apoptosis of the renal cells, sections were stained with a TUNEL (terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labelling) method. In this case, the ApopTag© Peroxidase *in situ* apoptosis detection kit (Chemicon International) was used.

Pilot Studies:

There were 5 groups in total in the preliminary studies. Group 1 is the control group (CTL, n = 3). As discussed this group underwent anesthesia and heparinization only. Urinary and blood sampling was conducted hourly for 4 hours after induction (Figure 1).

Group 2 underwent normothermic CPB for 4 hours after cannulation (BP, n = 3). Urinary and blood sampling was conducted hourly up to end experiment. This group received a mannitol infusion of 0.5 g/kg/hr from the time of initiation of CPB (Figure 2).

Group 3 underwent CPB for 1 hour after cannulation. Then the pump was turned off for 0.5 hours of normothermic circulatory arrest. Perfusion was then reinstated by turning the pump back on after which CPB was maintained for another 3 hours. This group received a mannitol infusion of 0.25 g/kg/hr from the time of initiation of CPB (LoM, n = 6, Figure 3).

Group 4 was identical to LoM apart from the mannitol dose which in this group was 2 g/kg/hr (HiM, n=7, Figure 3).

Group 5 underwent CPB with cooling to 18°C (nasopharyngeal) after cannulation (HCA, n = 6). Once this temperature was reached, DHCA was initiated for a total of 1 hour after which CPB with rewarming was resumed for 3 hours. Blood and urine sampling took place pre-CA, one hour after resumption of circulation and every hour thereafter to end experiment (Figure 4).

A histological comparison was conducted specifically between the HiM and LoM groups. A quantitative analysis was performed in a blinded manner at a magnification of 40x under the light microscope. The number of proximal convoluted tubules (PCTs),

number of PCTs with greater than 25% loss of brush border and number of PCTs with cellular debris were assessed for each field of view (5 random fields of view per slide).

Apart from calculating means and standard deviations to assist in interpretation of the data, no major statistical calculations were performed in the pilot portion of the study owing to the small numbers in each of the groups.

Final Protocol:

Eighteen (18) female pigs weighing 30-40 kg were randomized to undergo either BVP or CP in an otherwise identical protocol of anesthesia, cannulation, cooling with CPB, DHCA, resumed CPB and sacrifice (Figure 4). There were 9 animals in each group. These animals had a rectal temperature probe inserted as well and rectal temperatures were measured and recorded during CPB. After cannulation and initiation of CPB, intravenous mannitol, 0.5 g/kg/hr, was initiated and administered, by infusion throughout the experiment. The aorta was cross-clamped as described and cooling to 18°C (nasopharyngeal) was undertaken. The time to reach this goal temperature was recorded and CPB was maintained at goal temperature until 1 hr had elapsed from initiation of extracorporeal support. The circulation was arrested by termination of pump flow for 1 hour. Pump flow was then resumed and continued for 3 hours post arrest while the animal was rewarmed. The time taken to rewarm to 37°C was recorded. During both cooling and rewarming the temperature gradient between the water bath and the animal was equal to or less than 8°C. The animals underwent renal harvest and processing at end experiment as described.

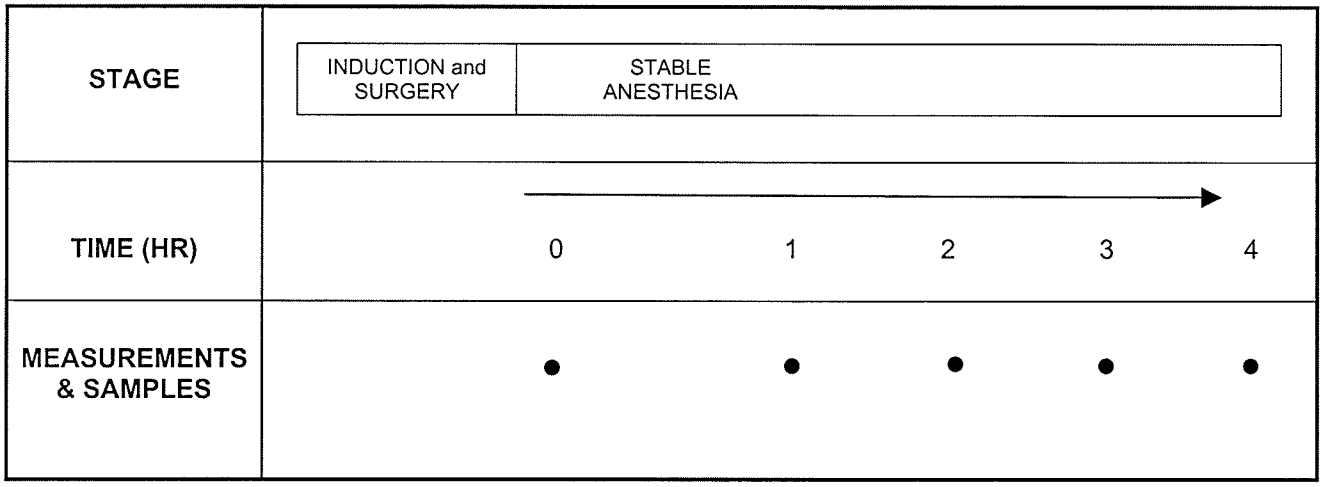

Figure 1: Protocol for CTL group (n = 3). Pigs induced and heparinized and maintained under anesthesia for 4 hours with blood and urine sampling as indicated (black circles).

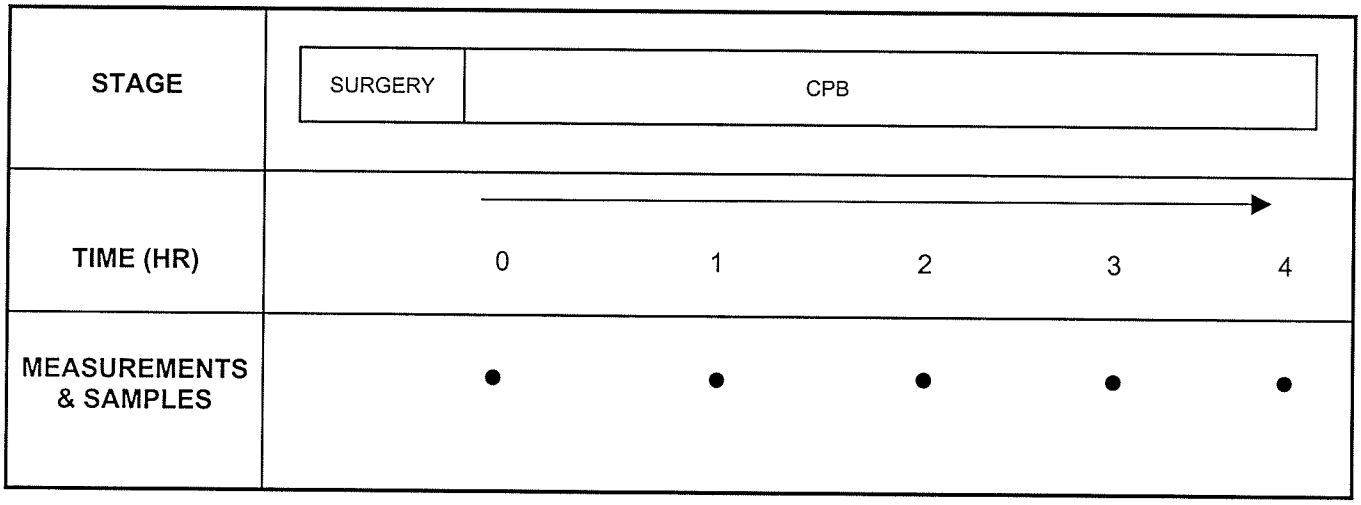

Figure 2: Protocol for CPB group (n = 3). Pigs induced, cannulated (surgery) and perfused with cardiopulmonary bypass (CPB) for 4 hours with blood and urine sampling as indicated (black circles).

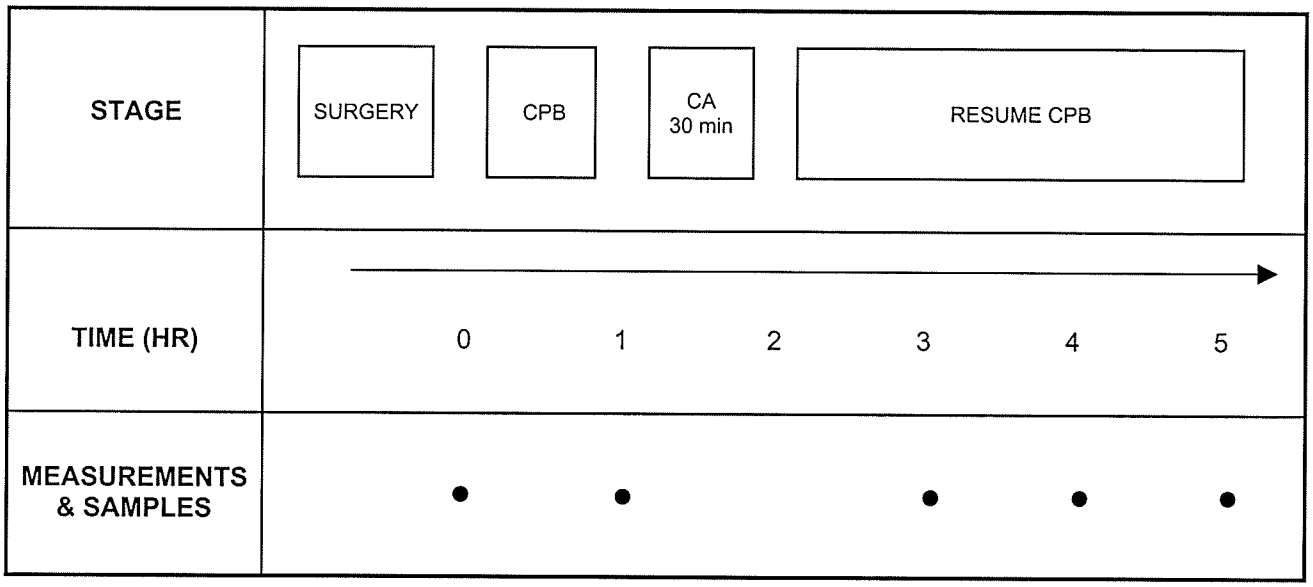

Figure 3: Protocol for HiM (n = 7) and LoM (n = 6)groups were identical (apart from mannitol dose). Pigs underwent 30 min of warm circulatory arrest (CA) after 1 hour of cardiopulmonary bypass (CPB). Post CA, CPB was resumed for 3 hours. Note the time gap between 1 and 2 in the figure represents 30 min; all other time intervals are as stated. Blood and urine sampling performed as indicated (black circles).

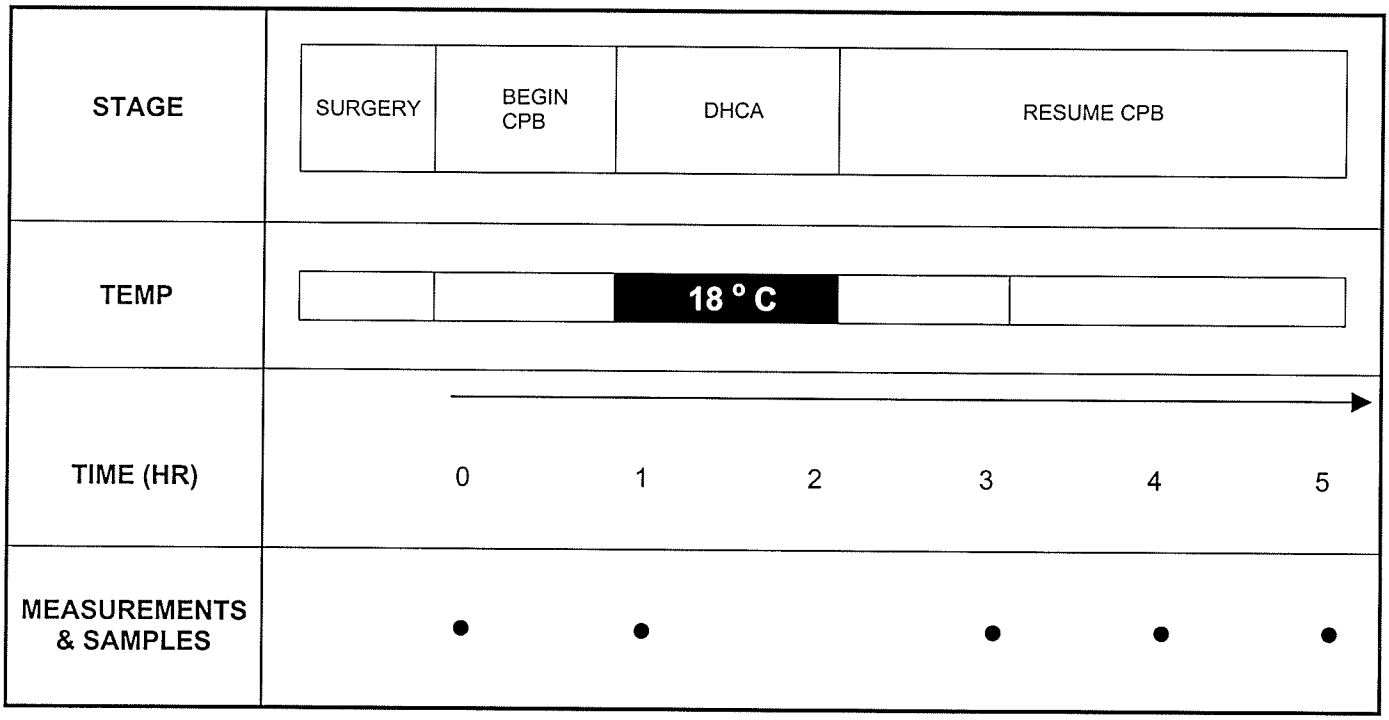

Figure 4: Protocol for HCA (n = 6), BVP (n = 9) and CP (n = 9)groups. All underwent the same protocol of cardiopulmonary bypass (CPB) with cooling to 18°C, followed by 1hour of deep hypothermic circulatory arrest (DHCA) and then 3 hours of reperfusion with warming to 37°C. Note that the HCA group from the pilot studies underwent DHCA immediately on reaching 18°C, whereas the BVP and CP groups in the final protocol were maintained on CPB at that temperature to ensure standardized duration of 1 hour of extracorporeal support prior to DHCA. Blood and urine sampling was undertaken as indicated (black circles).

Computer-controlled BVP: Once stable with pump flows of 3.0 to 3.5 L/min, MAP 60 to 65 mmHg, and PaO_2 >300 mmHg, animals in the final protocol were randomized to either BVP or CP, which was maintained to the end of the experiment.

The technique and software for institution of variable bypass has been previously described[52,53,55]. In brief, the target minimal MAP and maximum amount of computer-controlled modulation of the roller pump were set. A baseline mean pressure of 65 mmHg was targeted with a variation of 20 mmHg. At any time, roller pump rpm could be manually adjusted by rheostat control, though such adjustments were minimized. Figure 5 shows representative arterial pressure traces comparing conventional apulsatile perfusion and biologically variable perfusion.

Urine Proteomics: These studies were performed on animals in the final protocol. All urine was collected with the urometer in an ice bath. At each collection time period, decanted urine was immediately centrifuged at 500*g* for 7 min. The urine was subsequently frozen at -80°C until time of processing. Urine samples were thawed on ice, vortexed and centrifuged for 5 min at 10,000*g* to remove remaining cell particles. Five μL of urine supernatant were applied in duplicate to normal phase chips (ProteinChip NP20; Ciphergen, Freemont, CA). One μL of 35% Δ-cyano-4-hydroxycinnamic acid (CHCA: Ciphergen) was applied to each spot and air-dried. Chips were read using surface enhanced laser desorption/ionization time of flight mass spectrometry (SELDI-TOF-MS; ProteinChip Reader II: Ciphergen) in the positive ion mode with the following settings: laser intensity, 230; detector sensitivity, 6; detector voltage, 1700 V; 240 laser

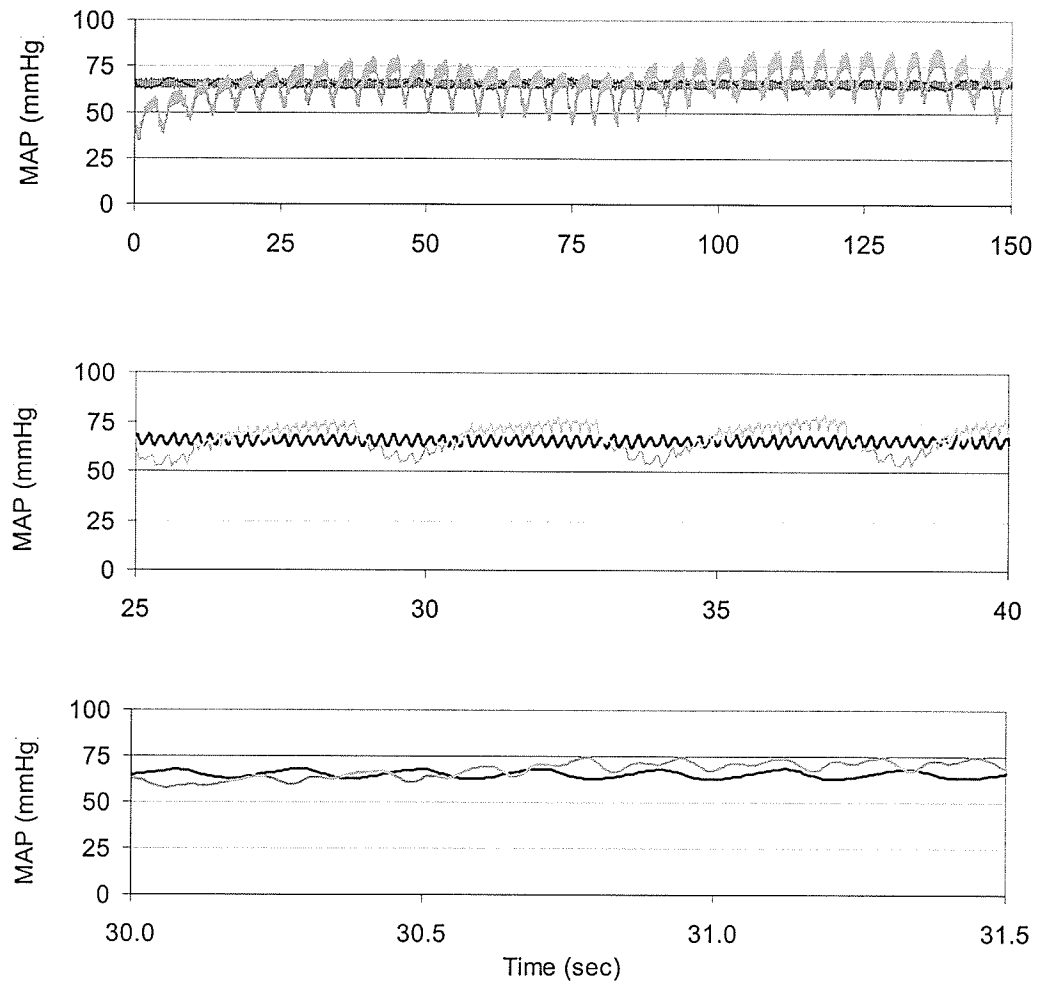

Figure 5: Representative femoral arterial pressure traces for pigs undergoing 1) conventional apulsatile perfusion (CP); black, and 2) biologically variable perfusion (BVP); gray trace. Time scales decrease by a factor of 10 with each trace from top to bottom panels. Mean blood pressure is identical for both traces (65 mmHg). Long-term oscillations of approximately 75 sec are evident with BVP in top panel as well as oscillations at 15/min – reflective of encoded breathing rhythm. In middle panel, 3 complete respiratory oscillations are seen. Small rhythmic

Figure 5 (cont'd): oscillations, best seen in the bottom panel are reflective of the beat-to-beat variation with individual stroke volume and instantaneous heart rate (in gray); different from the monotonous rectified sine wave typical of apulsatile bypass (in black).

shots were collected per sample. Peak labelling was performed using the ProteinChip Software (Version 3.1) for peaks with a signal to noise ratio of $\tau 3$ displayed for a mass over charge (m/z) ratio from 2 to 80 kD.

Statistics: For animals in the final protocol data are presented as mean ± SD unless otherwise noted. All continuous variables between the randomized groups were compared at baseline and at each time interval by a split-plot 1-way analysis of variance for repeated measures. Bonferroni's correction for repeated measures was applied to within group comparisons. When significant group or group × time interactions (G × T) were found, least mean square matrices were generated and within- and between-group comparisons were made. SAS 9.1 software was utilized for statistical analysis.

RESULTS

Pilot Studies:

There was no mortality in the 25 experiments. The hemoglobin and mean arterial pressure were stable among all groups, although markedly higher in the CTL group. Serum lactate increased similarly in the groups undergoing circulatory arrest following recirculation (Figure 6). Peak serum creatinine was slightly higher in the groups undergoing warm circulatory arrest (LoM and HiM groups; Figure 7).

The urine volume was highest in the HiM group, intermediate in the groups receiving a mannitol infusion and lowest in the LoM group (Figure 8). Notably, 3 of the animals in the LoM group were anuric for at least one hour post circulatory arrest.

Peak GST was elevated in all circulatory arrest groups, but markedly so in the HiM group, intermediate in the HCA group and much lower in the LoM group (Figure 9). The peak AP and GGT followed a consistent pattern with each other whereby they were high in the warm circulatory groups, intermediate in the HCA group and negligible in the other groups (Figures 10 and 11 respectively).

The most detailed histologic examination was carried out in the HiM and LoM groups to assist in the interpretation of the markedly different GST results between these groups. As seen in Table 1 this analysis showed that although both groups suffered the same degree of loss of brush border (38.6% vs. 46.4% respectively) there was markedly more cellular debris in the tubules of the LoM group (18.9% vs. 36.5%). This is representatively shown in Figure 12. The histology of the CTL and BP groups, as

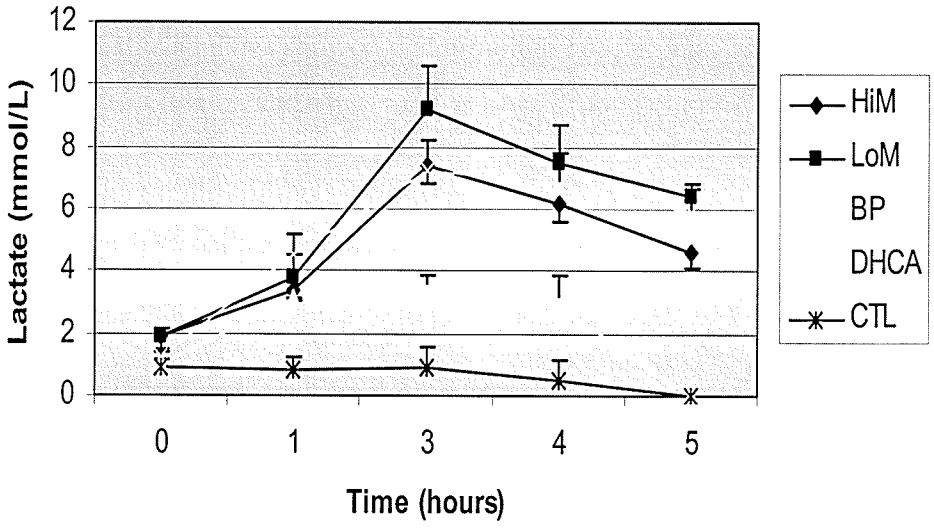

Figure 6: Mean serum lactate for pilot studies over course of experiments.

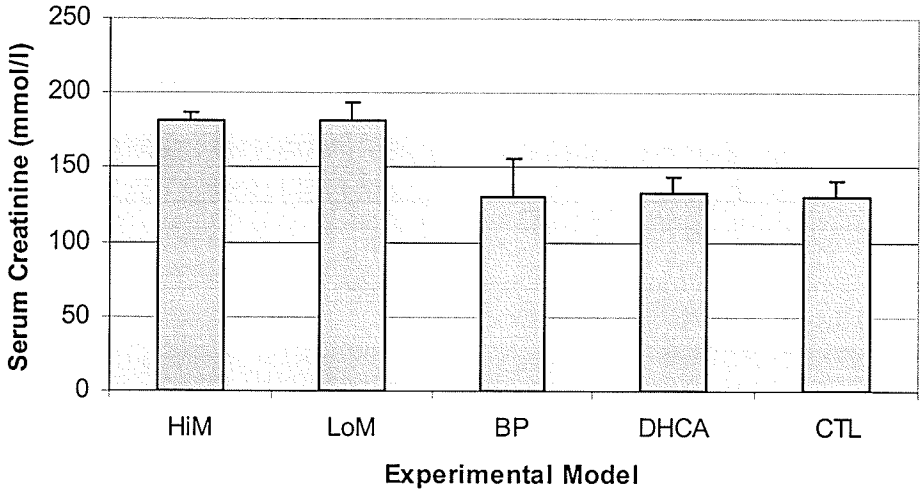

Figure 7: Peak serum creatinine observed in pilot studies.

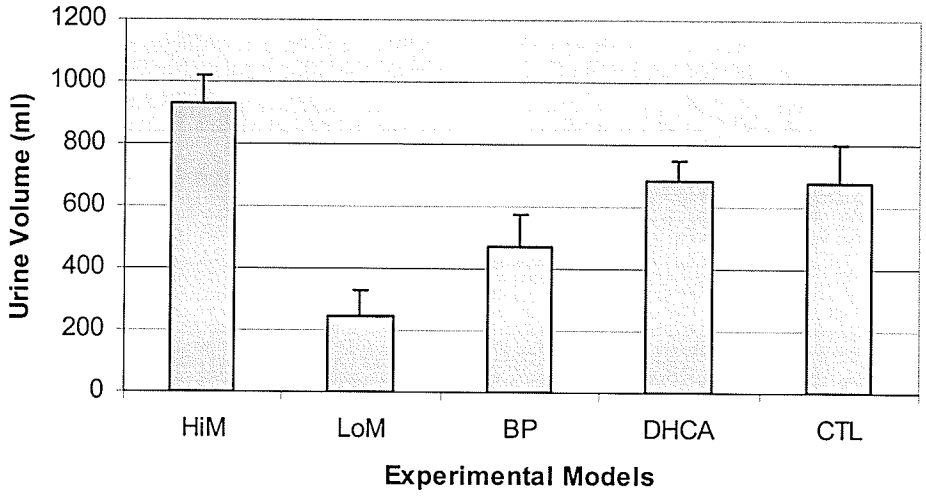

Figure 8: Mean total urine volume production during pilot studies. Note highest output in high mannitol group (HiM), lowest in the low mannitol group (LoM) and intermediate in all other groups receiving an intermediate dose of mannitol (BP, DHCA and CTL).

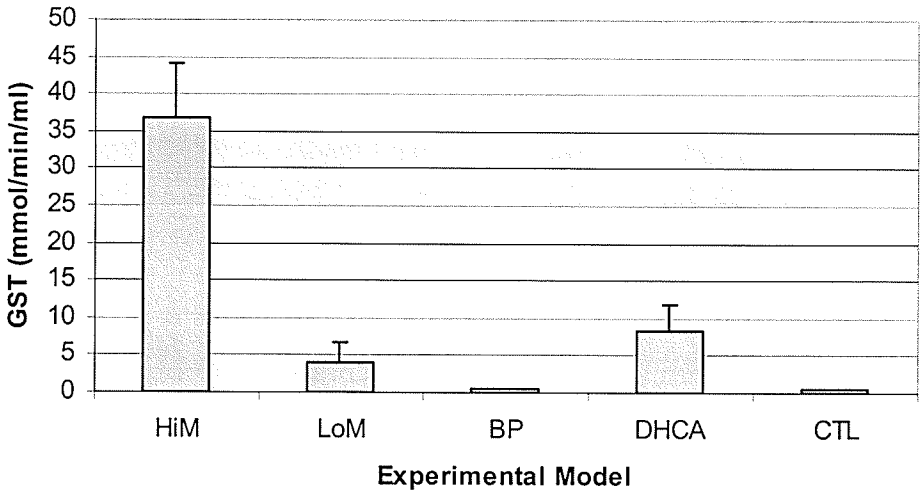

Figure 9: Mean peak GST activity seen in each of the pilot studies. Note the large discrepancy between the warm circulatory arrest groups (HiM and LoM) who underwent the same protocol apart from mannitol dose and the intermediate level seen in the hypothermic circulatory arrest group (DHCA).

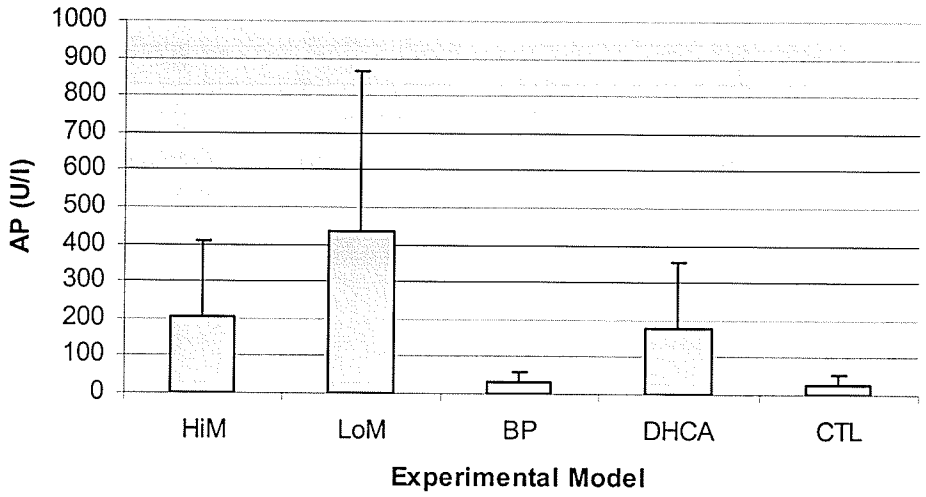

Figure 10: Mean peak AP concentration level seen in pilot studies. Note the highest levels seen in the warm circulatory arrest groups (HiM and LoM) followed by the hypothermic circulatory arrest group (DHCA).

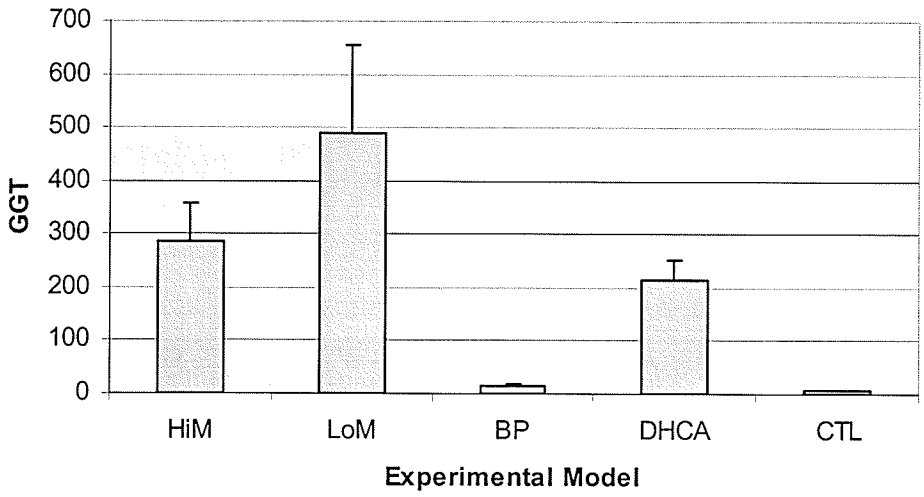

Figure 11: Mean peak GGT concentration seen in the pilot studies. Note that like the results seen for alkaline phosphatase, the highest values are in the warm circulatory arrest groups (HiM and LoM) followed by the hypothermic circulatory arrest group (DHCA).

Group	n	Number of Tubules	Cellular Debris*	%*	>25% loss Brush border	%
HiM	7	41.3 ± 6.6	7.5 ± 3.6	18.9 ± 10.3	16.0 ± 5.4	38.6 ± 10.3
LoM	6	38.4 ± 4.9	13.6 ± 6.0	36.5 ± 16.0	17.5 ± 4.5	46.4 ± 11.2

Table 1: Number of proximal tubules (PCTs), number of PCTs with cellular debris and number of PCTs with greater than 25% loss of brush border per field of view for the pilot groups undergoing warm circulatory arrest (HiM and LoM). * $p < 0.05$ between groups using Student's t test.

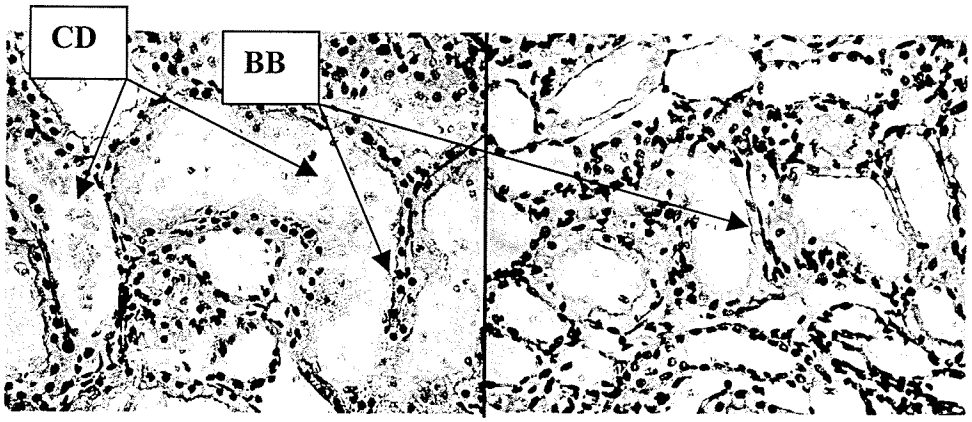

Figure 12: Periodic Acid Schiff Stains (PAS) of proximal tubules of warm circulatory arrest groups receiving low mannitol dose (LoM) and high mannitol dose (HiM) during pilot studies (40x light microscopy). Note that the there is significantly more cellular debris (CD) as shown by arrows in the LoM tubules. Both groups display loss of brush border (BB). Arrows demonstrate where patchy BB stained with PAS persists, although this intensely stained rim around the lumen of the tubules is lost in greater than 75% of the circumference demonstrating BB loss in these warm circulatory arrest groups.

qualitatively assessed by the pathologist was basically normal. The tissue of the HCA group was not analyzed.

Final Protocol:

Each of the 18 experiments was carried out successfully to completion. The temperatures and hemodynamics are shown in Table 2. Nasopharyngeal temperature was not different between groups at any time period as planned by protocol. After 1 hour of CPB with cooling, rectal temperature was cooler at 19.4±0.9 °C in the BVP group compared to 20.7±0.8 °C in the CP group ($p<0.0003$; G × T). MAP, PaO_2 and $PaCO_2$ did not differ between groups at any time period. The mixed venous oxygen saturation during CPB was higher in the BVP group post circulatory arrest compared to the CP group ($p<0.04$; G × T). Arterial pH (pHa) was significantly lower in the CP group in the third hour post circulatory arrest; 7.34±0.07 compared to 7.39±0.08 in the BVP group ($p<0.04$; between groups). Hemoglobin was greater in the CP group compared to the BVP group at all post arrest periods ($p<0.03$; G × T). The pulse pressure was 26.4±4.5 mmHg in the BVP group compared to 6.8±1.7 mmHg in the CP group ($p<0.0001$; unpaired t test).

The urine outputs in both groups rose markedly with initiation of mannitol infusion, with significant decreases following DHCA (Figure 13). Total urine output post DHCA was higher in the BVP group at 530±216 mL compared to 242±75 mL in the CP group ($p<0.002$; unpaired t test). This difference was most marked in the first hour post DHCA where the BVP group produced 250±129 mL compared to 114±66 mL in the CP group ($p<0.02$; between groups). The CP group demonstrated a more positive fluid

	Pre CPB	1 HR CPB (Pre DHCA)	1 HR Post End of DHCA	2 HR Post End of DHCA	3 HR Post End of DHCA
Temp (N)					
BVP	36.9 ± 0.5	18.1 ± 0.1 *	37.2 ± 0.4	37.2 ± 0.5	37.1 ± 0.2
CP	37.1 ± 0.2	17.9 ± 0.4 *	37.1 ± 0.1	37.1 ± 0.2	37.1 ± 0.1
Temp (R)					
BVP	37.1 ± 0.5	19.4 ± 0.9 *+	36.5 ± 0.6	37.2 ± 0.5	37.1 ± 0.3
CP	37.4 ± 0.3	20.7 ± 0.8 *	36.0 ± 0.7	37.1 ± 0.2	37.4 ± 0.1
MAP					
BVP	66 ± 8	64 ± 2	63 ± 4	66 ± 5	64 ± 5
CP	65 ± 7	65 ± 2	65 ± 1	65 ± 3	65 ± 2
PaO_2					
BVP	458 ± 60	461 ± 33	335 ± 67 *	321 ± 79 *	349 ± 26 *
CP	467 ± 43	461 ± 29	311 ± 16 *	314 ± 83 *	313 ± 42 *
$PaCO_2$					
BVP	41 ± 4	36 ± 4 *	34 ± 4 *	40 ± 6	38 ± 6
CP	41 ± 4	37 ± 4 *	33 ± 4 *	39 ± 4	40 ± 4
SvO_2					
BVP		100 ± 0	84.9 ± 4.0 +	83.0 ± 4.7 +	82.5 ± 3.1 +
CP		100 ± 0	80.7 ± 4.9	77.9 ± 3.8	77.6 ± 4.2
pHa					
BVP	7.45 ± .04	7.43 ± .05	7.40 ± .04 *	7.37 ± .06 *	7.39 ± .08 *+
CP	7.48 ± .05	7.44 ± .05	7.41 ± .05 *	7.37 ± .06 *	7.34 ± .07*
Hb					
BVP	10.2 ± 0.9	6.2 ± 1.1 *	6.9 ± 1.1 *+	7.0 ± 1.2 *+	7.0 ± 1.2 *+
CP	10.0 ± 0.7	6.5 ± 0.9 *	7.5 ± 0.8 *	7.6 ± 0.7 *	7.7 ± 0.8 *

Table 2: Temperature, hemodynamics and other physiologic parameters for biologically variable (BVP) and conventional apulsatile (CP) groups. Temp (N) = nasopharyngeal temperature (°C), Temp (R) = rectal

Table 2 (cont'd): temperature (°C), MAP = mean arterial pressure (mmHg), PaO_2 = arterial partial pressure O_2 (mmHg), $PaCO_2$ = arterial partial pressure CO_2 (mmHg), SvO_2 = mixed venous O_2 saturation (%), pHa = arterial pH and Hb = hemoglobin (g/dL). * = $p < 0.05$ within groups vs. pre CPB, + = $p < 0.05$ between groups.

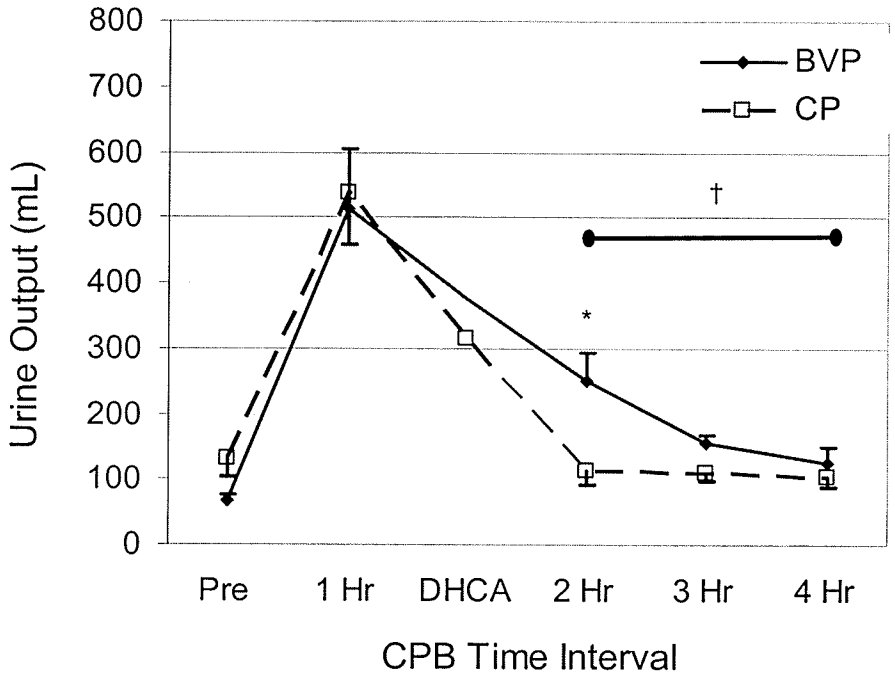

Figure 13: Mean urine output for BVP and CP groups during experiments. Note the significantly greater total urine output in the BVP group (solid line) post circulatory arrest (DHCA) most notably in the first hour post circulatory arrest compared to the CP group (dashed line). † = $p < 0.002$ (Student's t-test), * $p < 0.02$ (between groups).

balance of 1204±1414 mL compared to 612±866 mL in the BVP group though this difference was not statistically significant.

Urine protein to creatinine ratio was calculated for all time periods. The ratio was significantly higher in the CP group ($p<0.001$, G × T; Figure 14). Urinary enzymes GGT, AP, and GST concentrations were elevated in both groups but were markedly higher in the CP group compared to the BVP group at all time periods post DHCA (GGT, $p< 0.001$; AP, $p<0.0002$; GST, $p<0.002$; G × T). Peak levels of all urinary enzymes were seen at 1 hour post circulatory arrest. Peak GGT levels were 153±74 U/L in the CP group compared to 77±38 U/L in the BVP group (Figure 15). Peak AP levels were 123±49 U/L in the CP group and 66±28 U/L in the BVP group (Figure 16). Peak GST levels were 13.1±11.2 nmol/min/mL in the CP group versus 4.4±3.8 nmol/min/mL in the BVP group (Figure 17).

Confirmatory findings of abnormal urine proteins were observed with mass spectrometry. Pre DHCA there were few distinct peaks observed corresponding to a protein mass τ 7 kD. In contrast, both groups demonstrated the presence of new peaks at 8.5 and 9.8 kD post DHCA (Figure 18). Qualitatively these peaks decreased in amplitude with reperfusion as time progressed; however the decrease was visibly marked in the BVP group. By end experiment, 8 out of 9 animals in the CP group had persistent peaks at 8.5 kD defined as a signal to noise ratio > 1, compared to only 2 out of 9 animals in the BVP group. The 2-tailed Fisher's exact test had $p = 0.015$. The 9.8 kD protein signal was present in 7/9 animals in the CP group and 2/9 in the BVP group – Fisher's exact test; $p = 0.056$ (Figure 19).

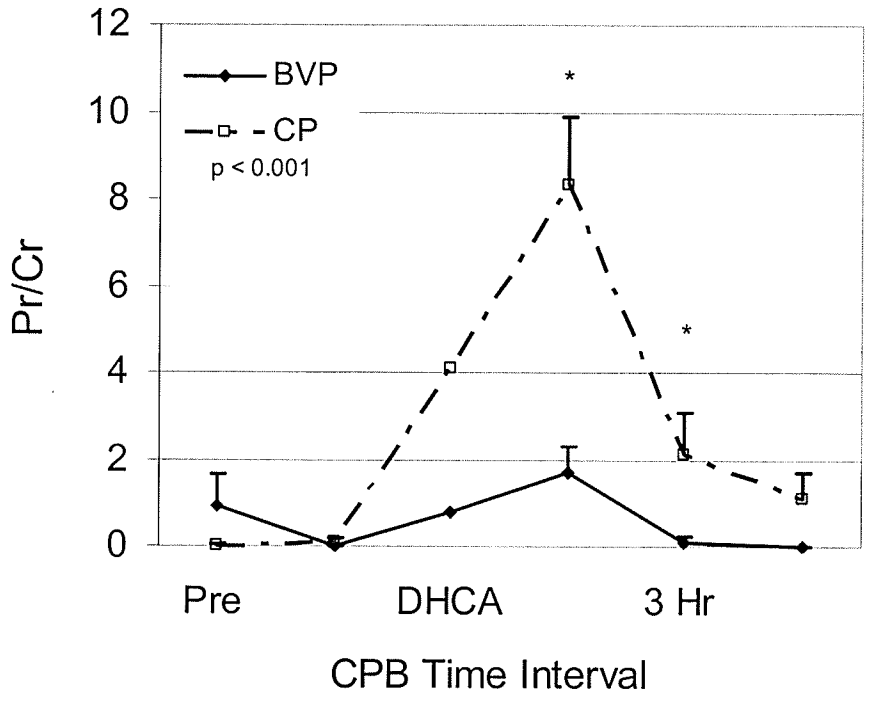

Figure 14: Mean urine protein to creatinine (Pr/Cr) ratio for CP and BVP groups during experiment. Note the much higher ratio in the CP group (dashed line) compared to the BVP group (solid line) at all time points post DHCA.

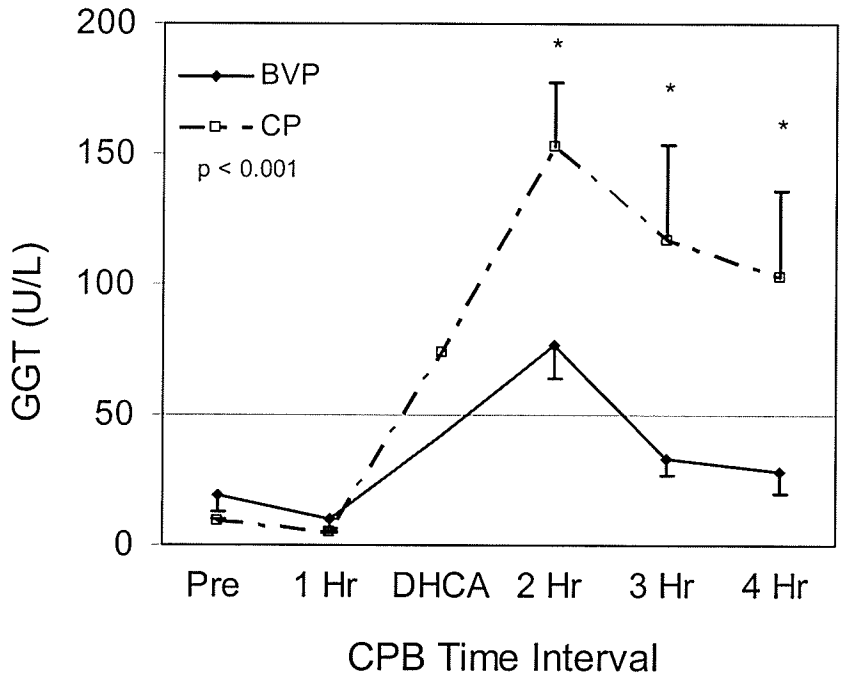

Figure 15: Mean gamma glutamyl transpeptidase (GGT) for CP and BVP groups during experiment. Note the much higher levels in the CP group (dashed line) compared to the BVP group (solid line) at all time points post DHCA.

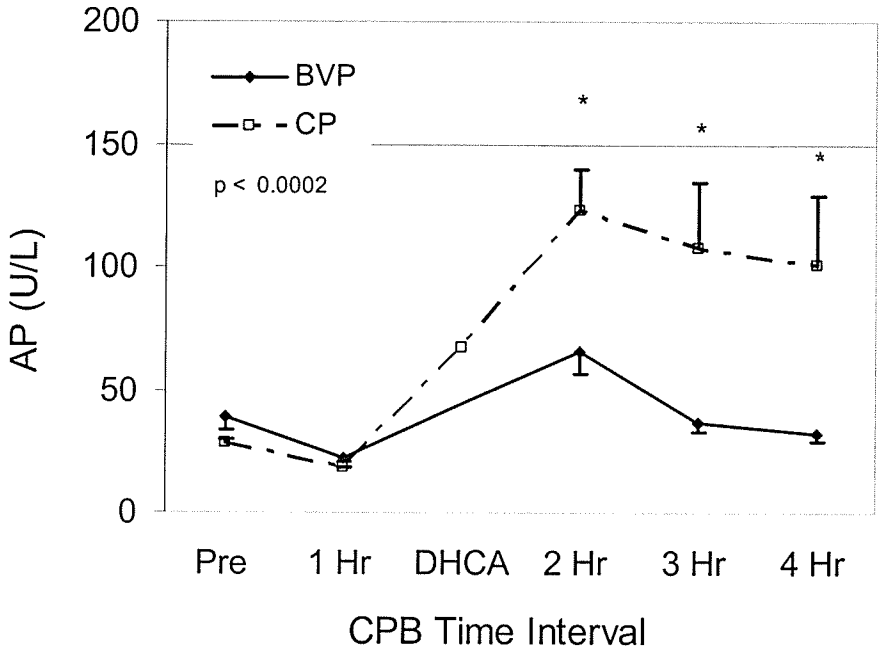

Figure 16: Mean alkaline phosphatase (AP) for CP and BVP groups during experiment. Note the much higher levels in the CP group (dashed line) compared to the BVP group (solid line) at all time points post DHCA.

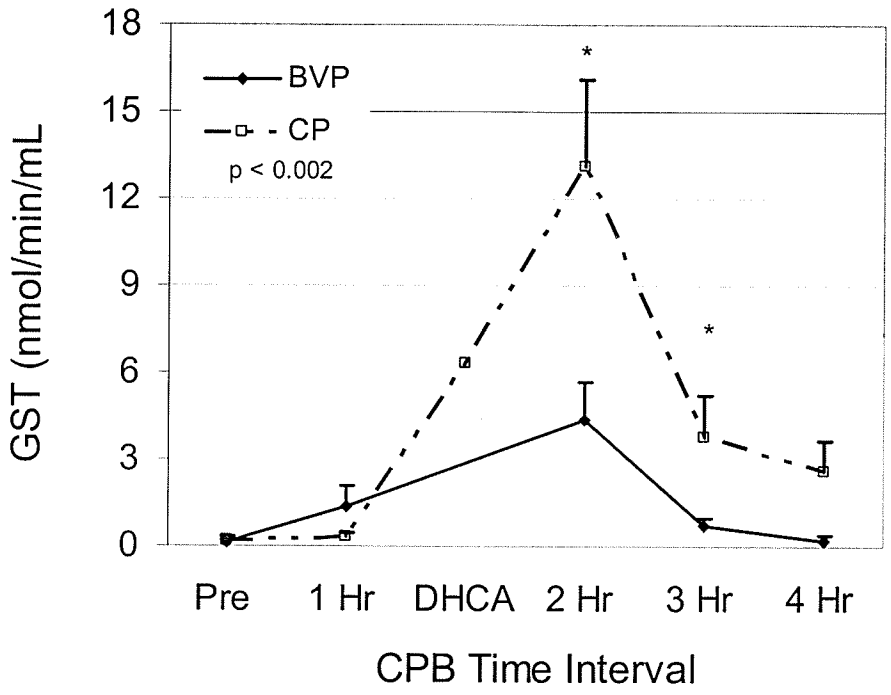

Figure 17: Mean glutathione s-transferase (GST) for CP and BVP groups during experiment. Note the much higher levels in the CP group (dashed line) compared to the BVP group (solid line) at all time points post DHCA.

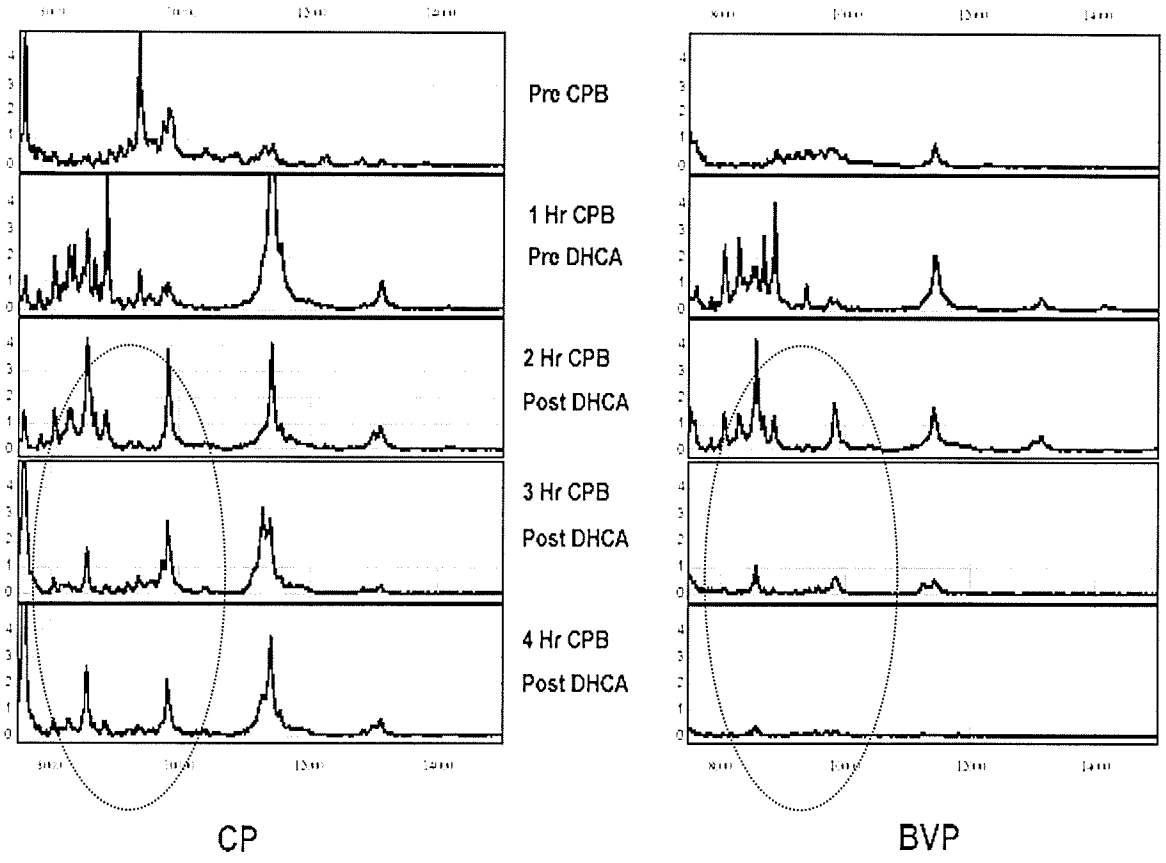

Figure 18: Representative urine protein spectra over time for one experiment in each group. Spectra were generated using surface enhanced laser desorption/ionization time of flight mass spectrometry (SELDI-TOF-MS). The time periods for measurement are shown between the two panels. Molecular weights are shown at the bottom of each panel. Note following DHCA there is more rapid attenuation of the newly arising peaks at the 8.5 and 9.8 kDa levels in the BVP group (in circles).

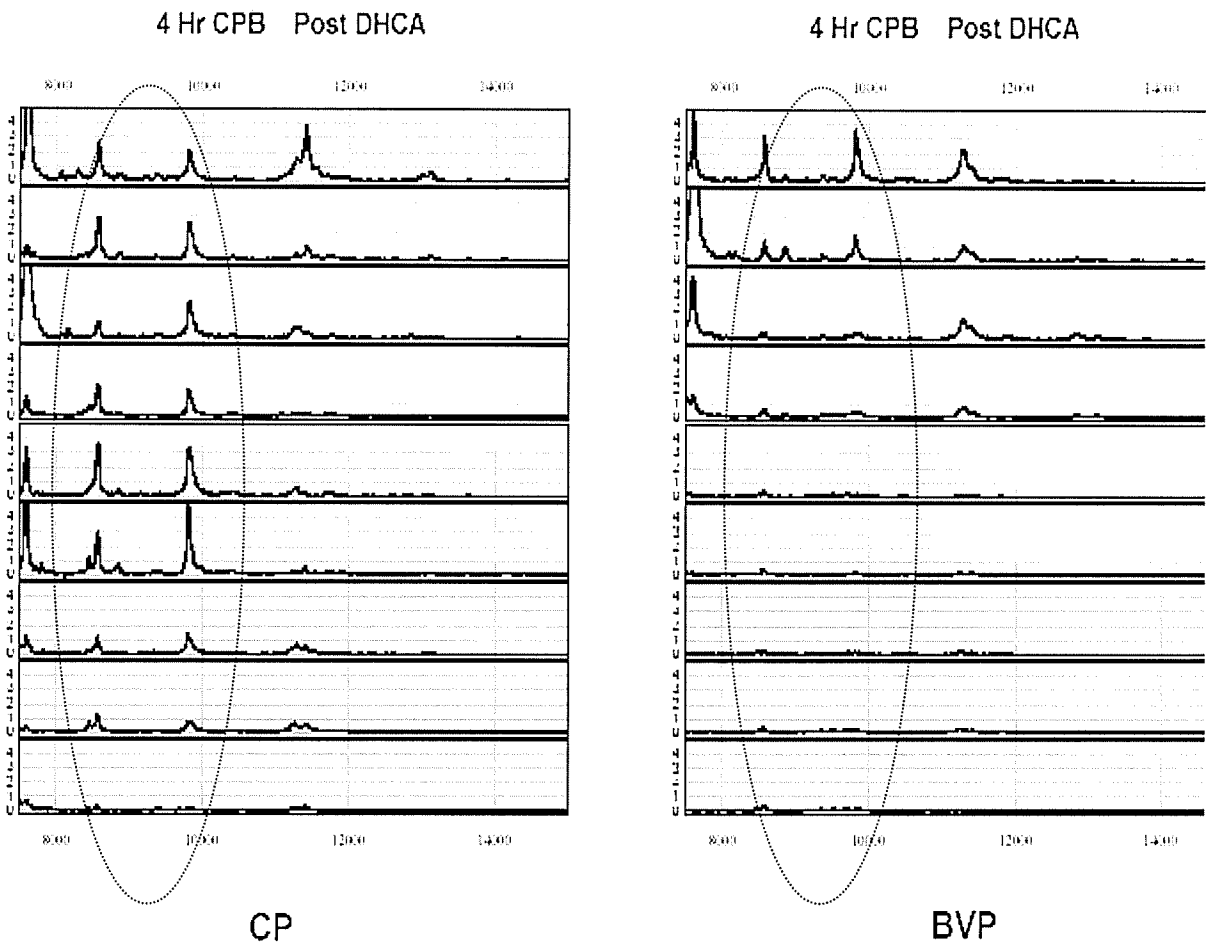

Figure 19: Protein spectrum for all animals in each group at end experiment. Molecular weights are shown at the bottom of each panel. Note that the CP group persistently demonstrates peaks of signal to noise ratio greater than 1 in 8/9 animals for the 8.5 kDa peak and 7/9 animals for the 9.8 kDa peak (in circle). This is in contrast to the BVP group in whom only 2/9 animals show persistent peaks at these weights (in circle).

The BVP group demonstrated more rapid cooling and rewarming with a mean cooling time of 21.0±9.0 min compared to 31.7±7.5 min in the CP group and a mean warming time of 22.1±3.9 minutes compared with 31.2±5.1 min in the CP group ($p<0.002$, unpaired t-test; Figure 20). The mean cumulative time saved for cooling and rewarming with the BVP approach was 19.8±11.2 min.

Qualitative assessment of the formalin-fixed, PAS-stained tissues under light microscopy did not demonstrate noteworthy necrosis of the renal parenchyma in either group. Furthermore, TUNEL staining did not show any difference between groups.

Serum aspartate aminotransferase (AST) was elevated in the BVP group during the experiment compared to the CP group ($p<0.012$; G × T). Peak levels were 140±46 U/L in the BVP group compared to 100±13 U/L in the CP group. In contrast, serum levels of alanine aminotransferase (ALT), AP, GGT, conjugated and unconjugated bilirubin, albumin and lactate were not different between groups suggesting no other difference in liver enzymes.

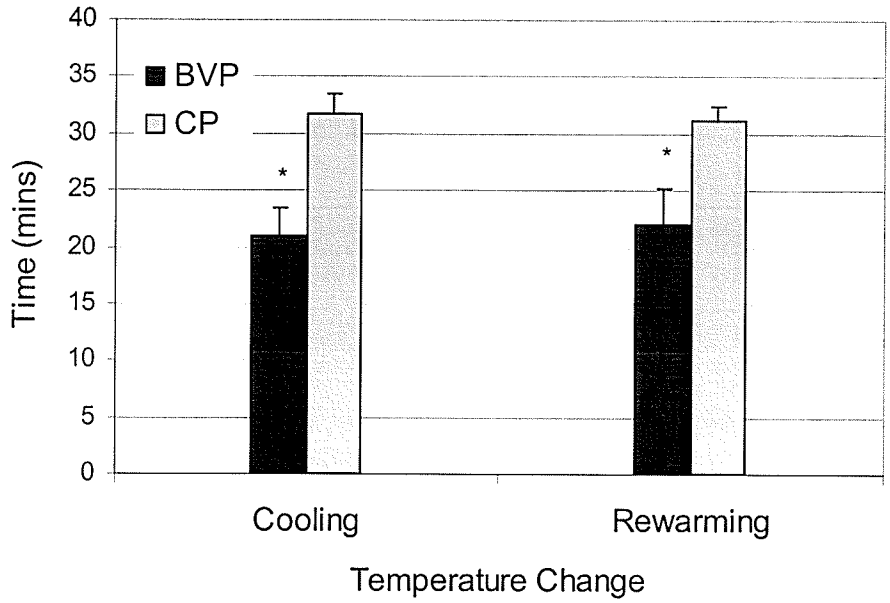

Figure 20: Mean cooling and rewarming times for the two experimental groups. Both cooling and rewarming were more rapid with BVP; * $p<0.002$ (unpaired t test).

DISCUSSION

BVP is a novel approach to address the limitations of non-physiologic flow of traditional apulsatile or more conventional pulsatile CPB. The fundamental principle underlying this adjunct to a life support device is the return of physiologic timing sequences to the roller pump signal – a restoration of biological noise seen with health. With either apulsatile or pulsatile CPB as conventionally applied, the variability of the beat-to-beat changes in heart rate, blood pressure and respiratory cycle are eliminated. Health is associated with a specific type of variation in each of these signals which have fractal temporal characteristic[34,35]. By way of example, sinus rhythm, breathing patterns, and beat-to-beat alterations in blood flow to organs are fractal in nature[40,41]. With BVP, the healthy fractal variation in the heart rhythm, fluctuations in pressure, and respiratory rhythm are all restored by computer-control of the roller pump output (see Figure 5).

The 25 pilot experiments carried out were used to design a final protocol to study the benefit of BVP relative to CP with respect to systemic perfusion and consequent renal dysfunction. The questions that arose in the determination of this final protocol were whether or not to use circulatory arrest and at what temperature, how to use mannitol and how best to assess the level of renal insult.

With respect to the protocol, it was clear looking at the urinary proteins and histology that the group undergoing CPB without circulatory arrest did not achieve a significant renal injury (Figures 9-11 respectively). Furthermore, the lactate increased mildly in this group, again suggesting that this duration of CPB in healthy young pigs did not result in a malperfusion syndrome that would be significant enough to use as a model for comparison between treatment groups (Figure 6).

Therefore, our preliminary results showed that to be able to demonstrate differences between groups, if at all, we would require a circulatory arrest model. All three of these groups (HiM, LoM and HCA) had a significant rise in GST, AP and GGT. The HCA group demonstrated the least renal damage. Histologically, renal tubular necrosis is represented early on by loss of the brush border[58]. Not surprisingly both HiM and LoM, the warm circulatory arrest groups, had significant loss of brush border and evidence of proximal tubular damage suggesting renal infarction (Table 1). In choosing a model we wanted a reproducible demonstration of renal injury, but not so severe that the ability to elucidate differences as a result of CP or BVP would be overwhelmed by the level of injury. As a result it seemed appropriate to use a model of DHCA which clearly caused more damage than the CTL and BP groups and yet not as severe as the warm circulatory arrest groups. As we would later see during histological assessment in the final protocol, the necrosis was quite limited in the DHCA group suggesting attenuated and therefore appropriate level of injury for this experiment. Finally, the clinical applicability of such a model is highly relevant because of its current use in cardiac surgery for children and in aortic arch replacement and selected neurosurgical cases in adults. DHCA in pigs at the specified temperature and duration has been studied previously so we are using a viable experimental model[5].

The use of mannitol with consideration of renal protection is controversial. In the pilot experiments, its use seemed to have a significant effect on urine output and presence of GST where the LoM group had a markedly lower GST result than the HiM group. This result was difficult to interpret. The urine output was significantly lower in the LoM group compared to all other groups (including transient anuria in some animals) and this

group, histologically, had significantly more cellular debris. It is possible that the wash out produced by higher doses of mannitol successfully cleared the GST during diuresis for easier detection. This is speculative and is not supported by the AP and GGT results; unless these brush border enzymes are more prone to loss than cytosolic enzymes such as GST. Importantly, we do not believe that the mannitol was responsible for higher GST levels as a result of nephrotoxicity because the degree of brush border loss between the HiM and LoM groups, as determined in a blinded fashion, was similar between these groups. As well the AP and GGT should have demonstrated the same trend as GST with diuresis, which was not observed. Finally, the literature suggests that addition of mannitol to the CPB prime does not exacerbate renal toxicity[59] and mannitol is routinely used in clinical practice. We, therefore, elected to continue with the use of mannitol infusion. Mannitol's clinical relevance, promotion of urinary output (allowing detection of urinary proteins which we are examining) and our strictly controlled infusion between experimental groups provided confidence in the soundness of this decision. In the future there may be opportunity to compare the two CPB strategies in the complete absence of mannitol as ability to detect early renal injury become more sensitive.

As a result we conceived a final protocol comprising the use of circulatory arrest at hypothermic temperatures to achieve a moderate renal insult, with use of a moderate dose of mannitol. This model allowed us to compare differences between animals randomized to BVP or CP as their perfusion strategy.

The results from this experiment support the contention that BVP, with its biologic or fractal variability, enhances systemic perfusion and minimizes renal damage compared to conventional apulsatile CP. Enhanced perfusion may be explained, in part,

by the convex relationship between flow (Q) and pressure (P) that occurs after a period of circulatory arrest, as demonstrated in the coronary circulation when flow is restored with cardioplegia delivery[60]. This relationship has been modelled as a power law such that $Q = P^a$, where 'a' is a constant. We have shown that when the flow-pressure relationship is convex (a power law curve is such a convex function), enhanced flow occurs at the same mean driving pressure with the addition of biological noise[55]. This seemingly paradoxical phenomenon can be generalized mathematically by applying Jensen's Inequality[61].

Overall urine output post DHCA was significantly higher in the BVP group. This difference was most marked in the first hour post DHCA. Although urine output is used as a surrogate of renal and systemic perfusion clinically, once again, extrapolation from the present study may be confounded by the use of mannitol. However, administration was strictly controlled between groups suggesting that the observed differences were truly related to perfusion strategy.

Renal injury results in proteinuria[62]. We applied several approaches to assess the appearance of proteins in the urine following DHCA, rewarming and ongoing perfusion with the two strategies for CPB. The most basic examination compared urine protein to creatinine ratio over time. A much greater ratio was seen in the CP group in the first 2 hrs post DHCA. This finding was buttressed when specific markers of tubular injury - GGT, AP and GST were examined. These urinary proteins, validated as sensitive early markers of renal tubular injury[56,57], were significantly elevated post DHCA at all times in the CP group compared to the BVP group. It has become increasingly apparent that enzyme markers can highlight renal tubular injury far earlier than more conventional indices such

as rising creatinine or diminished creatinine clearance. Mishra et. al[63] studied renal dysfunction following CPB in children using neutrophil gelatinase-associated lipocalin (NGAL) as an enzyme marker. This marker, identifiable within 2 hrs of bypass in children, correlated with >99% sensitivity and specificity to a 50% increase in serum creatinine from baseline. However, the increase in serum creatinine could only be detected following a delay of 24 – 72 hrs after surgery. This is consistent with our results where elevated enzymuria is seen in the absence of histologic changes or major differences in serum creatinine. Finally, an extremely sensitive approach to assess proteinuria is mass spectrometry urine proteomics - previously used to identify characteristic protein patterns with renal transplant rejection,[64,65]. In this experiment, the technique identified abnormal protein profiles in both groups following DHCA compared to baseline. The abnormal proteins persisted longer post DHCA in the CP group. Collectively, these results suggest BVP attenuated renal injury during reperfusion post DHCA.

Histological examination of the kidneys did not demonstrate any visible differences between groups with respect to acute tubular injury. This may reflect the relative insensitivity of histology in the detection of acute tubular injury, which in this experiment may have been below the threshold level for histological demonstration. The absence of distinct changes in cellular morphology is confirmed by TUNEL staining for apoptosis. The use of ELISA or other antibody related techniques, including immunohistochemistry for earlier apoptotic markers (e.g. caspase-3 activation), may help elucidate as yet unrecognized histological differences. At present, such techniques are not readily available for the swine proteome.

A beneficial effect of BVP on tissue perfusion was implied by a significant decrease in both the cooling and warming times. The BVP group required roughly two-thirds of the time to achieve target temperatures for cooling and rewarming compared to the CP group (Figure 20). To control for cooling duration, target temperature was maintained for a total of 60 min prior to DHCA, irrespective of the time to reach target. As well, despite difference in rewarming times, target temperatures were identical within 60 min of rewarming. However, the accelerated cooling and rewarming with BVP may have important clinical implications because duration of CPB is correlated to renal dysfunction[11,14,15]. While in this experiment, standardization of the protocol did not allow differences in CPB time; the cumulative time that would have been saved using BVP was 19.8±11.2 minutes. Furthermore, though cooling was referenced to nasopharyngeal temperature, measured rectal temperature was significantly lower in the BVP group by 60 min at target temperature. These findings imply better microcirculatory perfusion with more homogeneous heat transfer. The mixed venous oxygen saturation provides further evidence for enhanced perfusion in the BVP group. This group had higher mixed venous oxygen saturation than the CP group despite moderately lower hemoglobin concentration. Finally, the CP group showed greater acidosis in the third hour post DHCA compared to the BVP group with no difference in $PaCO_2$, indicating an increased metabolic acidosis with conventional perfusion.

By design, there were differences in pulse pressure between groups in this experiment. It could be argued that the observed differences between groups may relate solely to the higher pulse pressure in the BVP group. BVP was not compared to a monotonous pulsation strategy in this study. Our comparison to apulsatile CPB is still

clinically valid. This is because conventional, apulsatile, roller-pump CPB is the norm in most centres as a result of various ongoing controversies around pulsatility. Furthermore, in a previous experiment of CPB with moderate hypothermia, jugular venous oxygen saturation was greater with BVP than with monotonously pulsatile perfusion during rewarming. The mechanical ventilator, another life support device, improves gas exchange and respiratory mechanics when a biologically variable end-inspiratory pressure signal is added, compared to a monotonously delivered signal. Thus, we suggest that the improvements seen in the BVP group are due to biologically variability added as a form of physiological noise rather than the addition of monotonous pulsation alone. Finally, we did not attempt to characterize the variability pattern by a mathematical description known as 'energy equivalent pressure' which has been proposed by some groups to be the most accurate description of a pulsatile perfusion scheme[28,29,31]. This is readily applicable to monotonous but not variable strategies and there is ongoing controversy over whether this approach adds any real utility[29] so we did not feel it was necessary in the experimental analysis which had defined physiologic outcome measures.

The consistently higher hemoglobin concentration in the CP group was not expected given the strictly controlled fluid resuscitation. This group had a higher (though not statistically significant) fluid balance. It is possible that, given our findings suggesting poorer microcirculatory perfusion in this group, third spacing due to microcirculatory changes was increased in this group resulting in higher hematocrit with greater overall fluid balance. Post experimental body weight and analysis of peritoneal fluid are required to better understand these fluid shifts.

The finding of elevated AST in the BVP group was unexpected. This may imply decreased hepatic perfusion with BVP, which was contrary to our hypothesis and contradicts all other findings in this experiment. The difference was minimal with respect to hepatic injury and does not reach clinically significant levels. Furthermore, there were no significant differences in all other measured liver parameters that would corroborate hepatic injury.

The use of DHCA to initiate an ischemic insult may diminish how broadly these results should be interpreted in a clinical context. While these findings support BVP as a more effective strategy for microcirculatory perfusion, it would be helpful to demonstrate such benefits in a model without DHCA. Mass spectrometry urine proteomics is perhaps sensitive enough to permit study of a non-arrest CPB model, as are urinary markers of tubular injury such as NGAL. The use of deliberate whole body cooling is still considerable given that each year in the United States approximately 2,050 neonates undergo DHCA and 10,400 children greater than one month old undergo hypothermia with low flow[66,67]; as well as its use in a small proportion of adult cardiac or neurosurgery. The renal benefit and reduction in CPB time, associated with more rapid cooling and rewarming with BVP, would be noteworthy improvements to conventional management.

Future Directions: DHCA could be viewed as a model of renal transplantation in which the harvested organ undergoes the same process of cold circulatory arrest followed by reperfusion. This porcine model could be adapted to examine; i) harvested organ ischemia-reperfusion injury and, ii) biologically variable perfusion strategies to support

explanted transplant organs. Finally, if renal protection in a non-arrest model can be demonstrated, the potential benefit of BVP may be more pronounced clinically where the atherosclerotic burden and renal dysfunction could be expected to be greater than from results seen in healthy pigs. This approach to perfusion could also be considered for cerebral or visceral protection during aortic surgery and potentially improve performance of pumps used for extracorporeal membrane oxygenation (ECMO) or ventricular assist. Thus, many potential avenues for application of biologic variability to life support exist – a novel marriage of mathematic principles.

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