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# An Experimental Evaluation of Continuous Cardiotomy Reservoir Ultrafiltration

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**Abstract:** Ultrafiltration has been suggested as a means to reduce the morbidity associated with blood activation. However, the application of ultrafiltration to the highly activated blood of the cardiotomy suction subcircuit has not been investigated. The purpose of this study was to determine whether cardiotomy reservoir ultrafiltration (CRUF) would be effective in altering cytokine levels. Six swine, undergoing 90 min of cardiopulmonary bypass (CPB), were divided into two groups; one group was assigned to receive CRUF ($N = 3$), the other was to serve as controls and did not receive ultrafiltration ($N = 3$). Blood samples were analyzed for hematocrit, plasma-free hemoglobin, total protein, interleukin-8 (IL-8), and tumor necrosis factor alpha (TNF-α). Samples were taken pre-bypass, post-heparinization, every 30 min during CPB, post-CPB and post-protamine. All data were analyzed using a one-way analysis of variance (ANOVA), with significance accepted at $p < .05$. There were no significant differences found between treatment and control groups for plasma-free hemoglobin levels (22.4 ± 22.2 vs. 14.6 ± 14.4; 40.1 ± 26.1 vs. 40.0 ± 19.3). After 90 min of ultrafiltration, there was a significant decrease in TNF-α (261.6 ± 119.6 vs. 71.8 ± 11.4; $p = .02$). Although IL-8 levels decreased from throughout the experiment, concentrations did not reach statistical significance. In conclusion, CRUF can be used without increasing cellular destruction, and can decrease certain cytokine levels. Our results suggest that further clinical studies should be undertaken utilizing this technique with a larger sample size. **Keywords:** ultrafiltration, cytokines, cardiotomy reservoir, inflammation. *JECT. 2001;33:27–33*

The inflammatory response associated with cardiopulmonary bypass contributes significantly to the morbidity and mortality associated with cardiac surgery (1, 2). The pathophysiological consequences attributed to the activation of the inflammatory cascade include: a febrile response, leukocytosis, an increase in vascular permeability, acute respiratory distress syndrome, and multiorgan failure (3, 4). Reducing the inflammatory activation of blood has long been a challenge for clinicians utilizing extracorporeal circulation. Well recognized as a substantial blood activator, the cardiotomy suction return subcircuit has undergone relatively few modifications (5). The activating effects of cardiotomy suction have been attributed to a number of factors: air-to-blood mixing, contact with the cardiotomy tubing, and exposure of the blood to the pleural and pericardial spaces (6, 7).

Cytokines are humoral substances released in response to activation of the inflammatory system (8). These include tumor necrosis factor alpha (TNF-α), and interleukin-8 (IL-8). TNF-α stimulates neutrophils to adhere to endothelial cells and degranulate. TNF-α also induces a febrile response and the production of other cytokines, including IL-8 (9). IL-8 exerts its effects on neutrophils, causing an increase in production of neutrophil adhesion molecules. It also causes neutrophil degranulation, and is a powerful chemotaxic agent for neutrophils (9, 10). To attenuate the inflammatory response generated by these cytokines, clinicians have implemented the use of ultrafiltration to reduce the serum levels of these humoral substances.

Ultrafiltration is the process of removing water and low molecular weight solutes via a semipermeable membrane utilizing a hydrostatic pressure gradient and the process of

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convective transport (11). The efficacy of an ultrafiltrator is primarily determined by the diameter of the pores in the membrane. Within pediatric cardiac surgery, ultrafiltration has shown beneficial effects in removing inflammatory mediators (12–15).

However, the application of ultrafiltration to the highly activated blood of the cardiotomy suction subcircuit has not been described. Thus, the focus of this study was to determine if continuous cardiotomy reservoir ultrafiltration would be effective in altering cytokine levels found in the highly activated blood of the cardiotomy suction subcircuit.

## MATERIALS AND METHODS

### Animal Protocol

All animals used in this study received humane care in compliance with the Guide for the Care and Use of Laboratory Animals as published by the National Institutes of Health (NIH Publication No. 85-23, revised 1985). All subjects were anesthetized with a mixture of ketamine (20 mg $kg^{-1}$) and xylazine (2 mg $kg^{-1}$) administered intramuscularly. Maintenance anesthesia was achieved by the administration of pentothal (100 mcg $kg^{-1}$) and muscle relaxant pavulon (50 mcg $kg^{-1}$). Subjects were intubated and ventilated with a tidal volume of 20–30 mL $kg^{-1}$ and at a rate of 15–20 breaths per min. Electrocardiogram leads were placed to allow continuous monitoring of the heart rate. The femoral artery and vein were cannulated with 14-gauge needles for hemodynamic monitoring, medication infusion, and blood sampling.

In all subjects, a midline sternotomy was performed and the great vessels dissected free in preparation for cannulation. Before cannulation, the subject received a bolus dose of 400 iu $kg^{-1}$ of bovine lung heparin and adequate anticoagulation (greater than 480 sec) ensured via ACT analysis with kaolin-based assays. A purse string suture was placed in the aorta, and cannulation was achieved with a 7.0 soft flow arterial cannula (Terumo, Ann Arbor, MI). A second purse string suture was placed in the right atrium, and cannulation was achieved utilizing a 36/46 Fr dual stage cannula (Research Medical Inc., Midvale, UT).

After separation from CPB, the effects of heparin were reversed with protamine sulfate (1 mg protamine per 100 iu heparin administered). At the termination of each experiment, the animal was euthanized by the administration of sodium thiopental (4 mg $kg^{-1}$) and potassium chloride (20 mEq) directly into the aortic root.

### Cardiopulmonary Bypass

A standard cardiopulmonary bypass (CPB) circuit was utilized that consisted of a hollow fiber membrane oxygenator (Optima, COBE Laboratories, Arvada, CO), a soft shell venous reservoir (Edwards Lifesciences, Irvine, CA), a filtered cardiotomy reservoir (COBE Laboratories, Arvada, CO), an arterial line filter (COBE Laboratories, Arvada, CO), an ultrafiltrator (HC 1400 Maxi, COBE Laboratories, Arvada, CO), a centrifugal pump (Biomedicus, Medtronic Cardiopulmonary, Brooklyn Park, MN), and polyvinyl chloride tubing (Fig. 1). The circuit was primed with 2000 mL of Plasmalyte-A, 50 mL of 8.4% sodium bicarbonate, and 5000 U of bovine lung heparin. An in-line blood gas monitor (CDI 500, Terumo, Ann Arbor, MI) was used in both the arterial and venous line during the CPB procedure.

During CPB the following hemodynamic parameters were controlled: mean arterial blood pressure (50–80 mmHg) were maintained through the use of neosynephrine (100 mcg $mL^{-1}$) and sodium nitroprusside (200 mcg $mL^{-1}$), central venous pressure (0–2 mmHg), mixed venous saturation (50–70%), anticoagulation (activated clotting time greater than 480 sec), and hematocrit (20–25%).

Before initiation of CPB, the cardiotomy reservoir was isolated from the extracorporeal circuit, by clamping the tubing between the cardiotomy reservoir and the venous reservoir. The two field suckers were initiated at a rate of 200 mL/$min^{-1}$. Blood oozing into the surgical field was constantly aspirated to the cardiotomy reservoir during the experimental period. Five minutes after CPB was initiated, ultrafiltration of the aspirated blood commenced, the dedicated CRUF roller pump was set at a rate of 250 mL $min^{-1}$, pre- and postultrafiltrator pressures were monitored, and a 300 mmHg transmembrane pressure was held constant through the use of a variable restrictor. The ultrafiltrate volume was measured, and an equal volume of crystalloid solution was returned to the cardiotomy reservoir to maintain a constant volume. The cardiotomy volume was transferred to the venous reservoir in 200 mL increments, if the venous reservoir volume was inadequate to safely maintain CPB. All remaining cardiotomy volume was transferred to the venous reservoir just before weaning from CPB.

The blood sampling protocol followed the diagram in Figure 2. Blood samples were tested for hematocrit, total protein, as well as plasma levels of TNF-α, and IL-8. In addition, plasma-free hemoglobin levels were measured in baseline samples and postprotamine samples in all subjects. Plasma concentrations of TNF-α were determined with a specific enzyme-linked immunosorbent assay (ELISA) (Cytoscreen, Biosource International, Camarillo, CA). The sensitivity of detection for TNF-α was 6 pg/mL. The plasma level of IL-8 was determined by an IL-8/NAP-1 ELISA (Cytoscreen, Biosource International, Camarillo, CA). The sensitivity of detection for IL-8/NAP-1 was 6 pg/mL.

For ELISA tests, blood samples were drawn into laboratory red top tubes and stored on ice. The ELISA blood

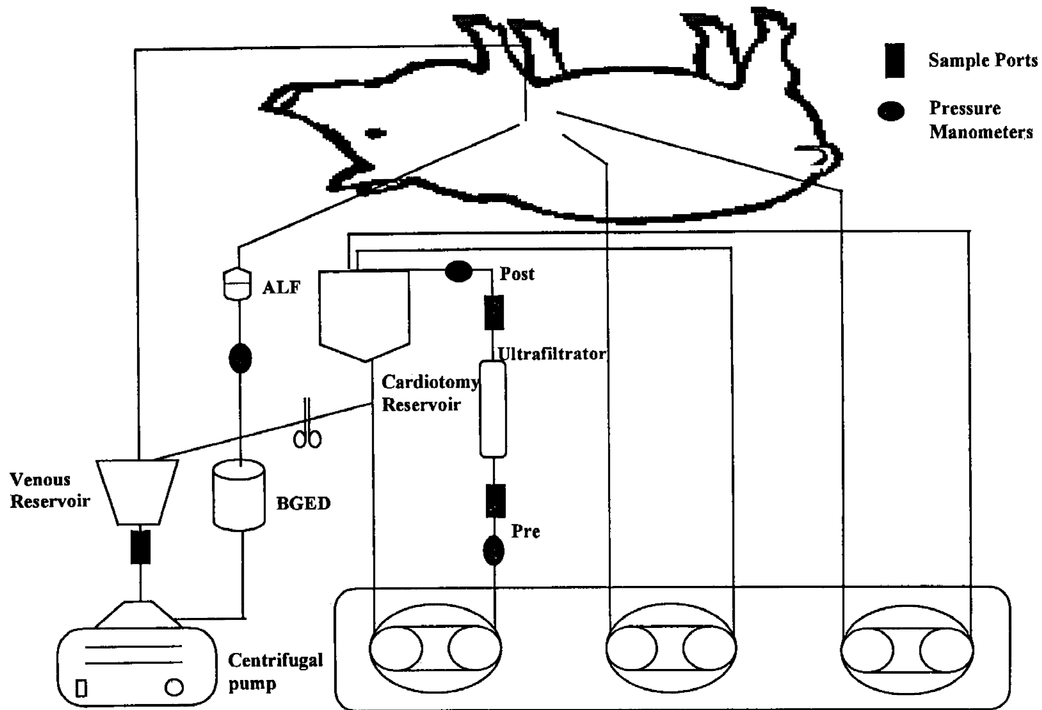


**Figure 1.** Experimental circuit diagram.

| | Baseline | Post Hep (5min) | On Bypass (5min) | 30 Min | 60 Min | 90 Min | Post Bypass (5min) | Post Pro (15min) |
|---|---|---|---|---|---|---|---|---|
| Femoral Arterial Catheter | * | * | * | * | * | * | * | * |
| Pre Ultrafiltrator | | | | * | * | * | | |
| Post Ultrafiltrator | | | | * | * | * | | |
| Venous Reservoir | | | | * | * | * | | |
| | | | | CRUF | | | | |

**Figure 2.** Blood sampling protocol.

samples were centrifuged at 4000 rpm for 15 min at 4°C. The plasma was stored in 2-mL aliquots and stored at −20°C for up to 60 days until the ELISAs could be completed.

**Statistical Analysis**

Data were collected and entered into a spreadsheet for analysis and were reported as mean ± standard deviation of the mean. Differences between the treatment and control groups were analyzed with one-way analysis of variance (ANOVA). Significant differences ($p < .05$) were further evaluated with a post-hoc test (Fisher's least significant difference).

## RESULTS

CRUF was performed without any complications during the experimental procedure. All of the subjects were

separated from CPB, and there were no perioperative mortalities. There were no significant differences between the CRUF group, and the control group in hematocrit levels at each time measurement (Fig. 3). There were no significant differences found between treatment and control groups for plasma-free hemoglobin levels (Fig. 4). No significant differences in any measured values were found between pre-ultrafiltrator and post-ultrafiltrator when corrected for hemoconcentration.

TNF-α levels were corrected for hemodilution and hemoconcentration using total protein levels. Arterial plasma levels of TNF-α were measured as a percentage change from baseline. TNF-α levels increased more than 6-fold in the control group during the experimental period; whereas, in the CRUF group levels of TNF-α stayed fairly constant. After 90 min of ultrafiltration, there was a significant decrease in plasma concentration of TNF-α within the cardiotomy reservoir (261.6 ± 119.6 vs. 71.8 ± 11.4 pg $mL^{-1}$; $p = .02$). Plasma concentration of TNF-α measured in the cardiotomy reservoir of the control group remained fairly constant with no reduction noted (Fig. 6).

IL-8 levels were corrected for hemodilution and hemoconcentration using total protein levels. Arterial levels of IL-8 were measured as percentage change from baseline, the control group consistently had a greater percentage increase from baseline than did the treatment group; however, this difference did not reach statistical significance (Fig. 7). Cardiotomy reservoir concentration of IL-8 was

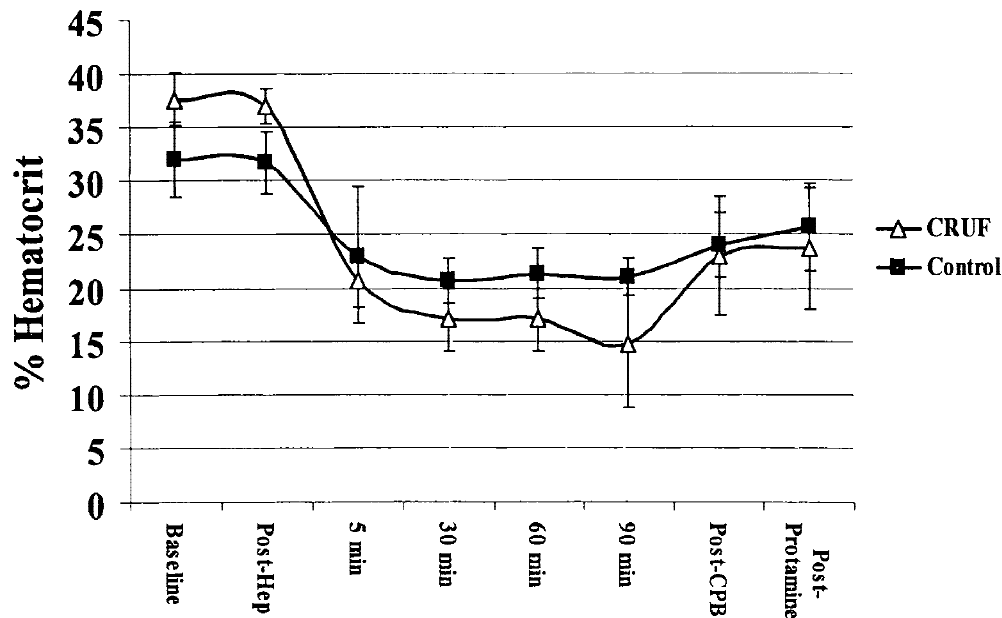


**Figure 3.** Changes in hemocrit during the experimental procedure.

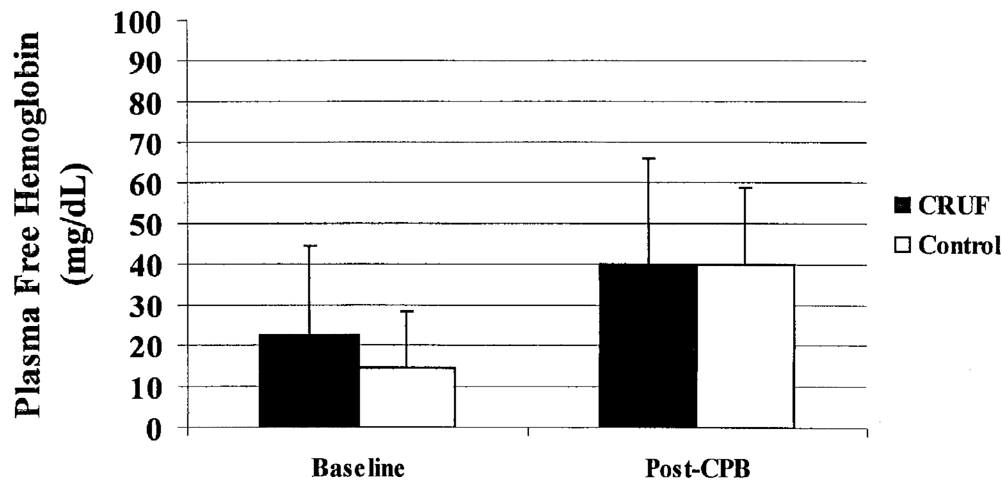


**Figure 4.** Plasma-free hemoglobin levels.

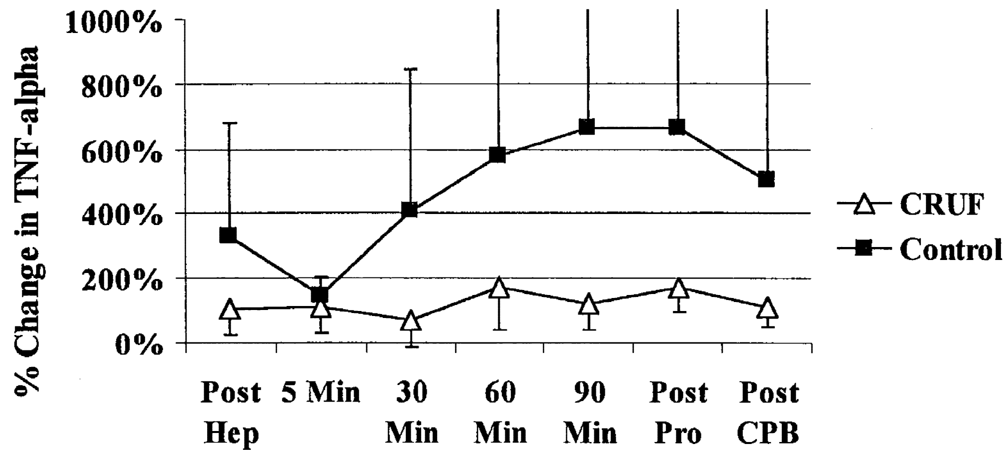


**Figure 5.** Percentage change from baseline in arterial TNF-α concentration.

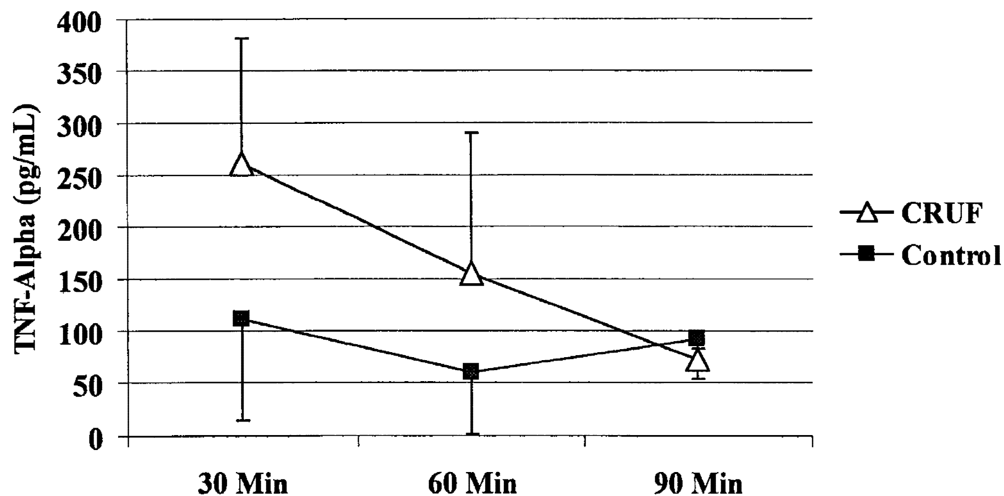


**Figure 6.** TNF-α levels measured in the cardiotomy reservoir.

consistently lower in the CRUF group than the control; however, this difference did not reach a statistically significant level (Fig. 8).

## DISCUSSION

The concept of removing inflammatory mediators to reduce the injurious effects of extracorporeal circulation has been examined extensively (12–16). A number of studies have shown that the removal of various humoral and cellular activated mediators reduced the vascular permeability seen in patients receiving extracorporeal circulation. The use of ultrafiltration has shown an increase in cerebral metabolic recovery, decreased cerebral edema, as well as an increase in cardiac and pulmonary function (17–19). Several methods of ultrafiltration have been utilized by clinicians during extracorporeal circulation. Conventional ultrafiltration (CUF) is performed during the rewarming period when complement and cytokine levels are increased (13, 20). Zero-balanced ultrafiltration (Z-BUF) is a modification of CUF in which large volumes of ultrafiltrate is removed during the rewarming stage of CPB, the volume removed is then replaced with an equal amount of crystalloid. Z-BUF has shown beneficial effects from the early removal of factors that activate the inflammatory response (21). Modified ultrafiltration (MUF) involves hemoconcentrating the patient's blood after CPB. Typically blood is removed from the aorta and returned to the right atrium. MUF has been shown to reduce the postoperative period length of stay, increase in hematocrit, and decreased plasma concentrations of inflammatory mediators (13, 14).

The technique examined in this study isolated the highly activated blood of the cardiotomy suction subcircuit and used a technique of dilutional ultrafiltration to remove inflammatory mediators. Detrimental effects of adding an ultrafiltrator to an extracorporeal circuit include the addition of 0.3 $m^2$ to 1.3 $m^2$ of blood activating surface area.

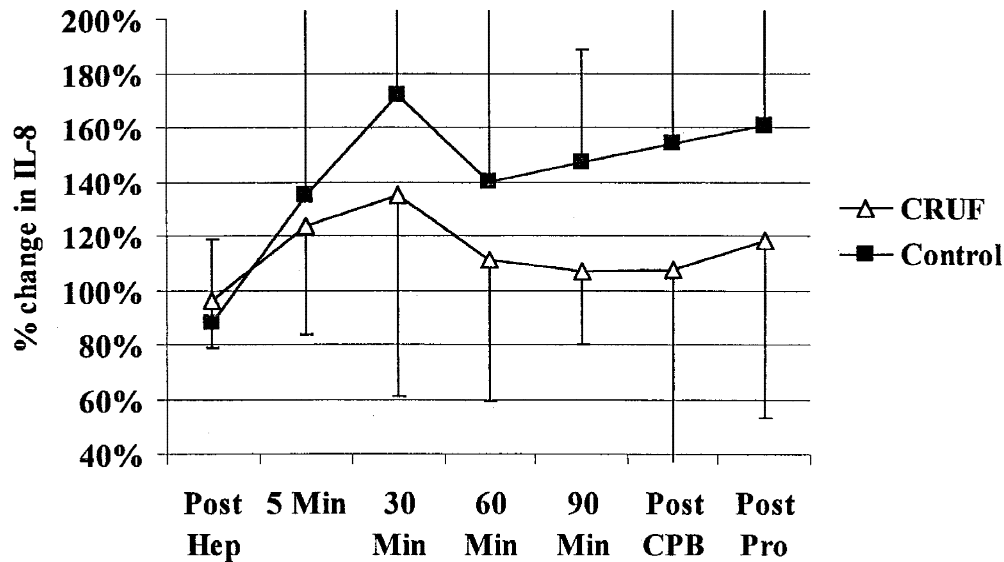


**Figure 7.** Percentage change from baseline in arterial IL-8 concentration.

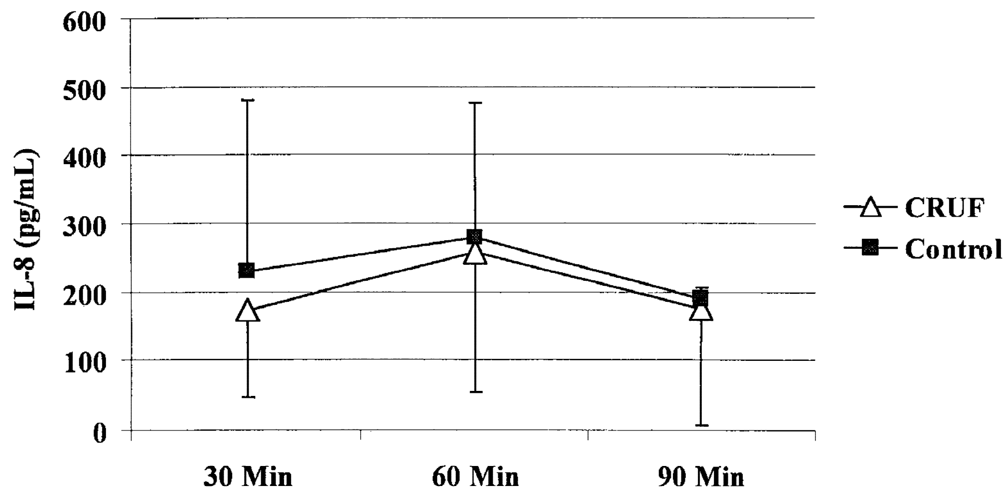


**Figure 8.** IL-8 levels measured in the cardiotomy reservoir.

Thus, the addition of an ultrafiltrator may induce the release of the very target substances that are implicated in injury. Another potential detriment is an increase in red blood cell trauma and release of plasma-free hemoglobin caused by increase in shear stress as the blood passes through the ultrafiltrator. Our results demonstrate that cellular damage, as measured by plasma-free hemoglobin levels, did not increase with the use of this technique. Cytokine levels were not significantly increased with the incorporation of the ultrafiltrator into the CPB circuit.

Our findings support those of others who found that TNF-α is readily removed with the use of ultrafiltration (13, 14). This removal may play a role in attenuating the production of other proinflammatory mediators. There is some controversy as to the effectiveness of ultrafiltration in removing IL-8 (14, 15, 22, 23). Our results support the findings of others as to the ineffectiveness of ultrafiltration in removing IL-8. Removal of early mediators of the inflammatory response may explain the decreased IL-8 concentrations seen in the treatment group.

Cytokine levels peak 2–4 hours postoperatively, this peak may have been missed in the present study, because the subjects were euthanized following the separation from CPB. Statistical significance was difficult to obtain with such a small sample size. Another factor influencing statistical significance is standard deviation, as with any in-vivo experiment, the inherent biological differences in response of the subject to the stimulus causes an increase in standard deviation. The removal of IL-10, which acts as an anti-inflammatory cytokine was not examined in this

present study. Removal of this substance may lead to an increase in postoperative morbidity associated with activation of the inflammatory cascade.

In conclusion, the results of our study demonstrate that CRUF can be performed without an increase in cellular damage. This technique is effective in reducing TNF-α levels found within the cardiotomy suction subcircuit. Further studies into this technique should examine the postoperative effects of this technique as well as determining the effects of CRUF on IL-10 levels.

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