

# The Effect of Volume Replacement on Serum Protein Concentration During Cardiopulmonary Bypass

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**Abstract:** Although controversy exists concerning the optimal total protein and colloid osmotic pressure that should be maintained during cardiopulmonary bypass (CPB), the primary volume expanders remain albumin and 6% hetastarch. The purpose of this study was to quantify the effect of adding boluses of volume replacement agents under various conditions to total serum protein values during CPB. A standard CPB circuit was utilized in eight 45-kg swine that had a priming volume (physiologic saline solution) of  $2309 \pm 245$  mL. Volumetric alterations occurred throughout the CPB period by the addition of combinations of physiologic saline solution, 6% hetastarch or 5% swine albumin. Pre- and postadministration samples were assayed for total serum protein, total protein, and albumin throughout the CPB period and at pre- and postvolume admin-

istration times. There was a significant decline in total serum protein with the initiation of CPB ( $6.14 \pm 0.49$  g/dL vs.  $3.40 \pm 0.43$  g/dL,  $p < .0001$ ). Addition of 12.5 g of swine albumin ( $N = 5$ ) to two different swine increased total serum protein significantly when compared to adding 500 mL of 6% hetastarch ( $N = 6$ ) (swine albumin  $12.4 \pm 6.3\%$  vs. hetastarch  $3.3 \pm 2.1\%$ ,  $p < .005$ ). A reduction in total serum protein occurred after hemodilution with varying amount of physiologic saline solution: 250-450 mL ( $7.4 \pm 4.5\%$ ), 451-650 mL ( $9.6 \pm 5.6\%$ ), and 651-1050 mL ( $19.4 \pm 4.0\%$ ). In summary, knowledge of total serum protein concentration and estimated circulating blood volume can be used to guide albumin and hetastarch administration following hemodilution. **Keywords:** albumin, colloid, edema, osmotic pressure. *JECT. 2001;33:227-232*

One technique associated with cardiopulmonary bypass (CPB) is hemodilution, which results in a large reduction in colloid osmotic pressure (COP), reportedly by 30-60% (1). Hemodilution results in a significant decrease in serum proteins, which leads to a significant reduction in COP and consequent weight gain during CPB (2,3).

COP is one of the primary factors in determining fluid flux across the capillary membrane, between the intravascular and interstitial space. The presence of excess non-permeable colloid maintaining COP contributes to the regulation of volume in the intravascular space (4). The amount of albumin transferred to the vascular compartment during CPB could be as much as 40% of the rapidly exchangeable pool, approximately 0.2 g/min (5). Therefore, maintaining a minimum COP is essential for adequate circulating intravascular volume (2).

One response in preventing a dangerously low COP from occurring is to increase the amount of colloid given as a maintenance fluid, by maintaining albumin levels it is possible to reduce the relative difference between the interstitial COP and the plasma oncotic pressure to a minimum. Perfusion practice is to add normal serum albumin when a large amount of crystalloid is added (6). However, this empiric practice may not maintain COP levels to within an acceptable range because of varying circulating blood volumes and volume status scenarios that are unique to each patient. The purpose of this study was to quantify the effects of adding boluses of crystalloid and colloid agents under various conditions to total serum protein values during CPB.

## MATERIALS AND METHODS

Eight swine  $45 \pm 5$  kg were placed on CPB to determine the effects of volume replacement on serum protein concentration during CPB. All eight animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No.85-23, revised 1985).

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### Anesthetic Management

Anesthetic technique as reported by Vang et al (7).

### Surgical Management

A midline sternotomy was performed and the great vessels dissected free. Each animal received a bolus dose of 300 IU/kg of bovine lung heparin and activated clotting times (ACT) reached a minimum of 400 seconds using kaolin-based ACT analysis prior to cannulation. A 7.0-mm arterial cannula (Texas Medical Products, Houston, TX) was placed in the aorta and secured with a purse string suture. A 34/46 dual stage venous cannula (Research Medical Inc., Midvale, UT) was placed in the right atrial appendage and secured with a purse string suture.

### Perfusion Management

The CPB procedure was instituted using a standard circuit including a hollow fiber membrane oxygenator (Optima, COBE Laboratories, Arvada, CO), a soft shell venous reservoir (COBE Laboratories, Arvada, CO), a filtered cardiotomy reservoir (COBE Laboratories, Arvada, CO), a 40 micron arterial line filter (COBE Laboratories, Arvada, CO), and a centrifugal pump (Biomedicus, Medtronic Cardiopulmonary, Brooklyn Park, MN), and polyvinyl chloride tubing. A CDI 500 (Terumo Sarns, Ann Arbor, MI) in-line blood gas monitor was used to monitor and maintain arterial blood gases, hematocrit (HCT), and venous saturation (SVO<sub>2</sub>) within normal ranges (pH 7.35–7.45, pC<sub>2</sub> 35–45 mmHg, BE –2.0 to +2.0, HCT > 20%, SVO<sub>2</sub> > 65%).

The circuitry was primed with 2309 ± 245 mL of physiologic saline solution (PSS), 10,000 IU heparin, and 50 mEq of sodium bicarbonate. No blood was used to prime the circuitry. Mild hypothermia of 32°C rectal temperature was achieved. 225 mL of red blood cells procured from autotransfusion were given when the hematocrit fell below 20%. Mean arterial blood pressures were maintained between 60 and 80 mmHg with the use of vasoactive substances (neosynephrine 80 mg/mL, Sodium nitroprusside 200 mg/mL) and the use of isoflurane. The flows were maintained between 1.6 and 2.4 L/min/m<sup>2</sup> to maintain blood pressure, arterial blood gases, and SVO<sub>2</sub> within stated ranges. After separation from CPB, heparin was reversed with protamine sulfate (1 mg protamine/100 IU total heparin administered).

At the termination of the experiment, the swine were euthanized with simultaneous administration of pentothal (1 mg/kg) and potassium chloride (20 mEq) directly into the aortic root.

### Experimentation

Volumetric alterations occurred throughout the CPB period by the random addition of plasmalyte A (300–1100 mL boluses), 6% hetastarch (500 mL boluses), or 5%

swine albumin (12.5-g boluses) through a quick prime line as volume was needed. Pre and post-volumetric alterations, arterial samples were drawn and assayed for total serum protein and HCT. HCT and total serum protein were also determined pre-CPB, one hour after initiation of CPB, and post-CPB. A spun HCT was performed in quadruplicate. The plasma was then collected for total serum protein analysis using the American Optical Refractometer (American Optical Company, Buffalo, NY). The refractometer was calibrated using distilled water and 0.9% NaCl.

Samples for total protein, albumin, and fibrinogen were collected at the following datapoints: pre-CPB, one hour after initiation of CPB, and post-CPB. These samples were sent to an independent laboratory where total protein and albumin concentrations were determined by an orthodiagnostic vitro-analyzer. A fibrometer measured fibrinogen concentrations with a mechanical clot-based test. Urine output was also recorded from intubation to termination of all swine.

### Statistical Analysis

Percentage change in total serum protein was calculated after the addition of plasmalyte, hetastarch, or albumin. The pigs were assumed to be similar with respect to starting blood volume.

The results of adding plasmalyte were evaluated using linear regression, where the measurements were assumed to be independent. The effects of hetastarch and albumin were compared to each other using Wilcoxon's rank sum test, and the measurements were assumed to be independent as well.

The validity of the total serum protein measurements from the refractometer was tested by comparison to the total protein laboratory findings. The lab values were compared to the experimental values using paired *t*-tests at each sample point.

Models were also designed for predicting total protein, albumin, and fibrinogen by using total serum protein values from the refractometer as the predictor variable. Time point was evaluated in each of the three models in its usefulness as a predictor (fixed effect).

The ratios of albumin to total serum protein and fibrinogen to total serum protein were examined at three time points (pre-CPB, 1 h post-CPB initiation, post-CPB). Analysis of variance (ANOVA) was used to determine if the ratios differ at any of the timepoints, while including a random effect for pig the measurement came from. The model was run separately for albumin and fibrinogen. Paired comparisons were made between timepoints when significant differences were found. All tests were two-sided, and a significance level of 0.05 was used. Data are presented as mean ± standard deviation of the mean.

## RESULTS

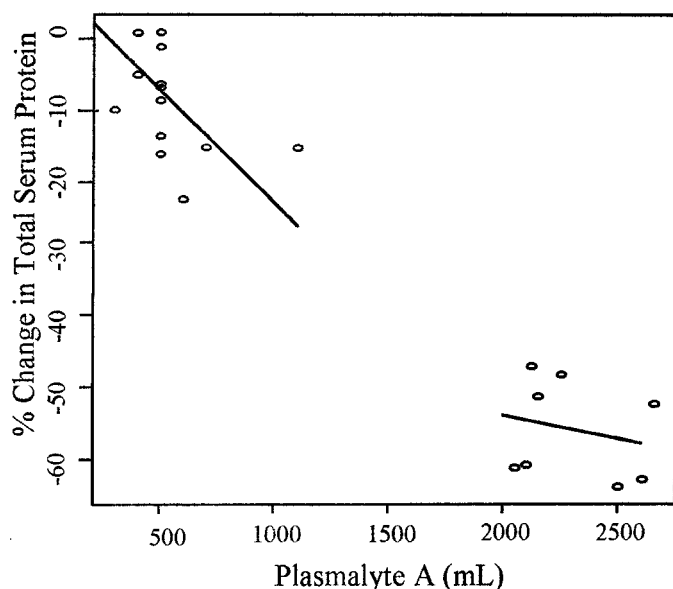
There was a significant decline upon initiation of CPB in total protein ( $6.14 \pm 0.49$  g/dL vs.  $3.40 \pm 0.43$  g/dL,  $p < .0001$ ) and total serum protein ( $5.90 \pm 0.51$  g/dL vs.  $2.60 \pm 0.445$  g/dL,  $p < .0001$ ).

From the regression analysis of percentage change in total serum protein on volume of plasmalyte given during CPB, it can be seen that as the volume of plasmalyte increases the percentage change of total serum protein decreases ( $p < .0001$ ) (Figure 1). The linear model that can be used to estimate the percentage change of total serum protein with volume of plasmalyte follows: % Change total serum protein =  $6.33 - 0.031 * \text{plasmalyte volume (mL)}$ .

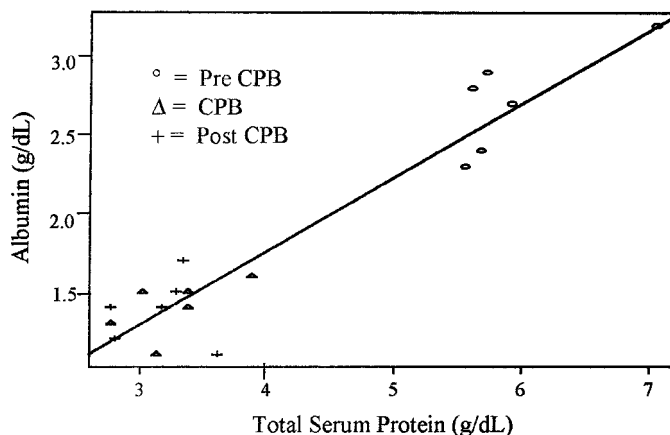
The plasmalyte volumes used to estimate this model range from 300 to 1100 mL, and estimates of percentage change in total serum protein should not be extrapolated from volumes beyond this range. The slope coefficient can be interpreted as: if the volume of plasmalyte is increased by 1 mL then the percentage change in total serum protein decreases, on average, by 0.031 (SE = 0.0046).

Six measurements of percentage change in total serum protein on six different pigs after the administration of 6% hetastarch (500 mL) produced a median percentage change of 3.3% (range = 0.6 to 5.6). Five measurements on two different pigs were taken after 12.5 g of swine albumin was administered. The median percentage change in total serum protein after albumin was 9.6% (range = 5.0 to 19.8).

Mixed effects regression models were developed to determine albumin levels (Figure 2). Two pigs were excluded



**Figure 1.** Effects of plasmalyte A on total serum protein.  $N = 13$  at volumes less than 1500 mL;  $N = 7$  at volumes greater than 1500 mL; CPB: cardiopulmonary bypass.



**Figure 2.** Albumin regression model from refractometer  $N = 8$  at each timepoint; CPB: cardiopulmonary bypass.

from this analysis, because they received albumin in the course of their treatment. The slope illustrates that if total serum protein is increased by 1 g/dL, then albumin is increased by 0.47 g/dL (Figure 2). The standard error for the slope (0.47) is 0.037 with  $p < .0001$ : albumin (g/dL) =  $-0.12 + 0.47 * \text{total serum protein (g/dL)}$ .

The analysis to determine if albumin changes with corresponding changes of total serum protein also excluded the two pigs that received albumin in the course of their treatment. From the analysis, the ratio was found to not differ significantly from pre-CPB, one hour post CPB initiation, or post-CPB ( $F = 2.18$ ,  $df = 2, 10$ ,  $p = .16$ ). Significant variation between pigs was found ( $F = 5.05$ ,  $df = 5, 10$ ,  $p = .014$ ).

Results prior to CPB indicate that total protein values were on average 0.24 (SD = 0.22) higher than TSP measurements ( $p = .019$ ). At the time of CPB, there was not a significant difference between total protein and total serum protein measurements (difference = 0.14, SD = 0.27,  $p = .19$ ). There also was not a significant difference post-CPB (difference = 0.23, SD = 0.84,  $p = .47$ ).

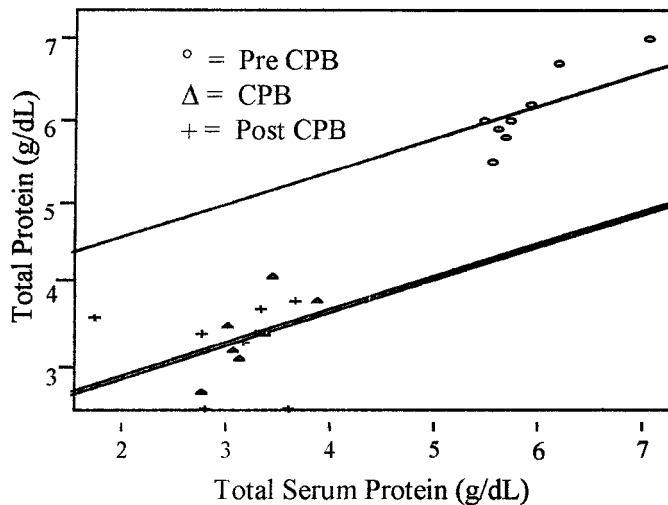
Total serum protein as determined by the refractometer was also used to predict total protein levels. Three separate fitted lines are displayed in Figure 3, and the regression results are as follows:

Pre-CPB: total protein (g/dL) =  $3.78 + 0.40 * \text{total serum protein (g/dL)}$

CPB: total protein (g/dL) =  $2.10 + 0.40 * \text{total serum protein (g/dL)}$

Post-CPB: total protein (g/dL) =  $2.06 + 0.40 * \text{total serum protein (g/dL)}$

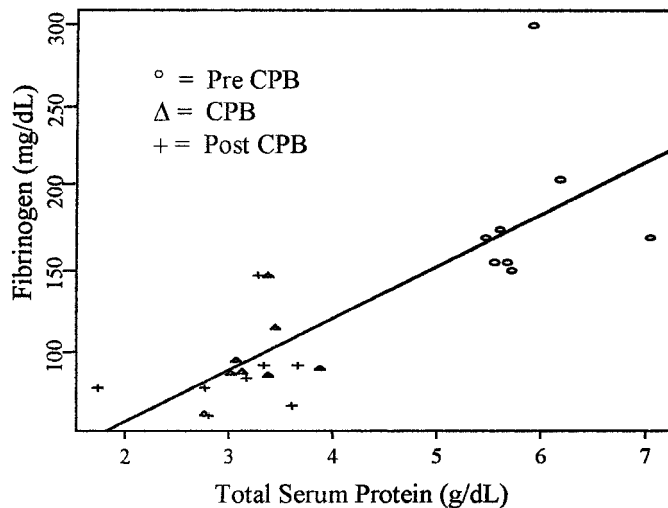
Regression analysis was also used to predict fibrinogen concentrations from total serum protein values (Figure 4). From the slope, it can be determined that as TSP is increased by 1 g/dL, FIB is increased by 31.7 mg/dL (Figure 4). The standard error for the slope is 5.22, and  $p < .0001$ :



**Figure 3.** Total protein regression model from refractometer.  $N = 8$  at each timepoint; CPB: cardiopulmonary bypass.

fibrinogen (mg/dL) =  $-5.99 + 31.68 * \text{total serum protein (g/dL)}$ .

The same type of analysis was performed to determine if fibrinogen levels change with total serum protein. From the analysis the ratio was found to differ significantly from between at least two of these time points pre-CPB, one hour post CPB initiation, or post-CPB ( $F = 5.52$ ,  $df = 2$ ,  $14$ ,  $p = .017$ ). Significant variation between pigs was also found ( $F = 32.27$ ,  $df = 7$ ,  $14$ ,  $p < .0001$ ). Since there was an over-all difference between the timepoints, a paired comparison was conducted to find where the differences exist. Table 1 illustrates that the mean ratio of fibrinogen to total serum protein decreases between timepoints, and a significant difference exists between pre-CPB and post-CPB.



**Figure 4.** Fibrinogen regression model from refractometer  $N = 8$  at each timepoint; CPB: cardiopulmonary bypass.

**Table 1.** Ratio of fibrinogen to total serum protein.

	Mean Ratio	Pre-CPB	CPB	Post-CPB
Pre-CPB	0.030	Same	0.093	0.0051*
CPB	0.028	0.093	Same	0.15
Post-CPB	0.027	0.0051*	0.15	Same

\* = Significant  $p$ -value  $< .05$ ; CPB: cardiopulmonary bypass.

## DISCUSSION

This study was performed to quantify the effects of adding boluses of crystalloid and colloid agents under various conditions to total serum protein values during CPB. Quantitative methods in a swine model were used to determine these effects. Knowledge of total serum protein values provides guidance when selecting and administering replacement volume.

Although research has suggested that significantly reducing COP has no correlation with adverse outcomes (1,8), several have illustrated the importance of maintaining minimum levels (9,10). A study by Schupbach and colleagues demonstrated that fluid retention is significantly correlated with plasma COP and albumin level in all tissues, except the liver (8). Mehlhorn concluded that increasing the COP of normothermic blood cardioplegia minimizes myocardial edema, thus preventing post-CPB cardiac dysfunction (9). Other studies have also shown increasing length of stay and an increase in morbidity and mortality in the critically ill with COP below 15 mmHg (10,11).

Both Kerkoff and Morissette reported normal adult COP in the critically ill patients to be approximately 21 mmHg with edema likely at a COP less than 15 mmHg (12,13). Morissette et al. concluded that in critically ill patients if COP decreased to less than 16 mmHg survival was jeopardized (13). Furthermore, if COP was 10 mmHg or less, the prognosis was poor, and the cardiopulmonary failure was likely to be followed by death. A reduction in the COP may lead to shunting, acidosis, as well as pulmonary and tissue edema (4,14). Progressive respiratory insufficiency after operation using CPB is characterized by an increasing fluid accumulation in the pulmonary tissue, with consecutive deterioration of pulmonary gas exchange (15).

The ability to maintain albumin concentrations within desired ranges also has significant value. A retrospective study of 857 critically ill patients by Blunt et al. stated that nonsurvivors had significantly decreased albumin concentrations as compared to survivors (11). Several studies performed in intensive care units (ICU) have reported increases in blood loss, weight gain, ICU and hospital stay, days on a ventilator, and even the development of new infections with decreased serum albumin concentrations (16,17). A prospective study of 7735 middle-aged British

men by Phillips and colleagues illustrated that serum albumin concentrations of 4 g/dL resulted in a mortality for 23/1000 per year, while a mortality of just 4/1000 per year existed when serum albumin concentrations were equal to or above 4.8 g/dL (18). Phillips et al. concluded that the strength of association between serum albumin and mortality to be comparable with that for cigarette smoking (18). The albumin level in the cardiac surgery patient associated with increased morbidity and mortality is generally between 2.5–3.5 g/dL (19–21).

The cardiac surgery patient on CPB will undergo hemodilution, reducing their circulating albumin and total protein concentrations, resulting in a 30–50% decline in COP (22,23). Our findings support these estimations, as total serum protein was significantly reduced upon initiation of CPB due to hemodilution. Once on CPB, the deleterious effects of hemodilution become proportionally more significant as total serum protein values could approach levels associated with morbidity and mortality. One explanation for this could be the exchangeable pool of albumin from extravascular to intravascular. Beattie et al. estimated that 40–70 g of albumin is exchangeable, and the amount transferred to the intravascular space during CPB may be 40% of this rapidly exchangeable pool (5). His report stated a mean net rate of albumin influx to be 0.2 g/min.

Colloid administration in our study consisted of albumin and hetastarch. In contrast to plasmalyte volume, boluses of both colloid agents significantly increased total serum protein. This study indicated albumin to be the superior agent for this purpose, as total serum protein was increased three times more with albumin administration than with hetastarch. Along with the addition of these agents once on CPB, numerous investigators have studied the effects of priming with albumin and hetastarch (24–26). The ability of albumin to preserve platelet function and reduce platelet deposition when added to the prime has been documented (24,25).

Despite knowing the effects that crystalloids and colloids can have on total serum protein during CPB, the information is of little clinical benefit if the values cannot be monitored in a simple, timely, and cost-effective manner. A clinical refractometer's ability to offer these attributes has been documented in previous studies (1,27). Although this study's intent was not to determine COP based on total serum protein measurements by a refractometer, as Beshere and Blackwell have done, it is consistent with their finding that the clinical refractometer can provide meaningful insight to help better manage patients on CPB (1,27). The total serum protein values measured by the refractometer consistently reflected the total protein values measured by the orthodiagnostic vitro-analyzer throughout the CPB period and post-CPB. The slightly higher total protein values compared to the total serum

protein values in the pre-CPB period could be a reflection of what each value has actually measured. The refractometer is designed to measure total serum protein, which does not include fibrinogen and anticoagulant that are measured in plasma samples.

The regression models utilized in this study are of importance, because they represent the possibility to predict total protein, albumin, and fibrinogen concentrations with the use of a simple device that may already be in the operating room. These concentrations may then be quantitatively manipulated to reach desired values based on the clinician's judgment of which critical levels to maintain. The ratio of fibrinogen to total serum protein was the only monitored variable that was not consistent throughout the entire surgical procedure. One explanation for this could be that a type of subclinical coagulopathy took place throughout the course of the CPB period. Although it was not manifested clinically throughout the experimental period, a discrete amount of fibrinogen may have been converted to fibrin, thus leading to a decreased amount of fibrinogen in proportion to total serum protein.

Although this study demonstrates relationships between total serum protein, total protein, albumin, and fibrinogen, additional studies are necessary before they can be applied clinically. Limitations in this study include the inherent differences between the swine model and the human patient. Discrepancies such as circulating blood volume and variations in pharmacological dynamics and kinetics must be addressed in future research. A small sample size and lack of isolated controlled versus experimental subjects were also limitations.

In summary, knowledge of total serum protein concentration can be used to guide albumin and hetastarch administration following hemodilution with crystalloid. The work data presented shows that when CPB volume is supplemented with a crystalloid solution, then total serum protein declines. However, this decline can be attenuated with the administration of colloids, with albumin being the most effective.

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