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Gravity Separation of Pericardial Fat in Cardiotomy Suction Blood: An In Vitro Model

M. Rhett Kinard II, BS, CCP; Anthony G. Shackelford, MHA, CCP; Joseph J. Sistino, MS, MPA, CCP

Division of Cardiovascular Perfusion, College of Health Professions, Medical University of South Carolina, Charleston, South Carolina

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Abstract: Fat emboli generated during cardiac surgery have been shown to cause neurologic complications in patients postoperatively. Cardiotomy suction has been known to be a large generator of emboli. This study will examine the efficacy of a separation technique in which the cardiotomy suction blood is stored in a cardiotomy reservoir for various time intervals to allow spontaneous separation of fat from blood by density. Soybean oil was added to heparinized porcine blood to simulate the blood of a patient with hypertriglyceridemia (>150 mg/dL). Roller pump suction was used to transfer the room temperature blood into the cardiotomy reservoir. Blood was removed from the reservoir in 200-mL aliquots at 0, 15, 30 45, and 60 minutes. Samples were taken at each interval and centrifuged to facilitate further separation of liquid fat. Fat content in each sample was determined by a point-of-care triglyceride analyzer. Three trials were conducted for a total of 30 samples. The 0-minute group was considered a baseline and was compared to the other four times. Fat concentration was reduced significantly in the 45- and 60-minute groups compared to the 0-, 15-, and 30-minute groups ($p < .05$). Gravity separation of cardiotomy suction blood is effective; however, it may require retention of blood for more time than is clinically acceptable during a routing coronary artery bypass graft surgery.
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Emboli have been a concern for perfusionists since the early development of cardiopulmonary bypass (CPB). Neurologic deficits have been noted in >50% of patients undergoing cardiac surgery (1), as well as lung dysfunction (2). Advancements such as cell salvaging, filters, and improvements in perfusion and surgical techniques have served to minimize the embolic load for cardiac surgical patients; however, neurologic injuries are still prevalent. Although several types of emboli have been suspected, fat has become a prominent subject for study.

Fat emboli are generated in several places during surgery and transferred to the CPB circuit through cardiotomy suction. Dissection to the sternum at the beginning of the surgery exposes large fatty tissue beds, a concern in the growing number of patients who are clinically obese. These subcutaneous fat stores, which are comprised primarily of oleic acid, have been identified as playing a key role in acute respiratory distress syndrome (3). Another source of fat emboli is the sternotomy. The sternum contains red bone marrow, which is ~5% fat (4) and is a significant source of emboli. This has been noted as an increase in blood lipids during the first postoperative day (5). Fat dripping into the pericardial well is also transferred to the CPB circuit with blood from surgical bleeding during dissection to the heart. Electrocautery also amplifies the amount of fat that drips into the pericardial well. Blood lipids such as cholesterol and triglycerides are transported mainly bound to proteins, helping to emulsify the cholesterol and triglycerides (2). However, unprocessed cardiotomy suction blood has been proven to increase the amount of cerebral lipid emboli (6).

Postoperative effects of fat embolization include brain tissue ischemia and lung dysfunction, which cause atelectasis and acute respiratory distress syndrome (2). Neurologic deficits are usually classified as stroke, type I injury, or diffuse brain damage, type II injury, and are suspected to be caused by various types of emboli. Murkin (7) suggested

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Address correspondence to: Joseph J. Sistino, MS, MPA, CCP, Division of Cardiovascular Perfusion, College of Health Professions, Medical University of South Carolina, 151B Rutledge Avenue, Charleston, SC 29425. E-mail: sistinoj@musc.edu
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that fat microemboli play a large role in type II injuries, including memory deficits and intellectual dysfunction. Small capillary and arteriolar dilatations (SCADs) are evidence of fat embolization in the cerebral arteries and have been identified to be a result of both CPB and cardiotomy suction blood (6,8).

Autotransfusion and commercial filters have been evaluated to determine their effectiveness in fat removal. Although results have been promising for autotransfusion and filters (9,10), these methods could result in additional costs where the techniques are not the standard of practice. Unfortunately, studies have also shown negative results for some autotransfusion devices and filters (9,11).

Spontaneous density separation or gravity separation of fat from blood is a technique studied by Engstrom and Appelblad (12). This involves cardiotomy suction blood settling undisturbed for an allotted time to allow for separation of fat from blood by density. The red blood cells settle at the bottom of the volume of blood and the fat separates to the top, reducing the embolic load of suction blood before it is given to the patient.

The purpose of this study was to evaluate an in vitro clinical model of withholding cardiotomy suction reservoir blood by using gravity separation to reduce lipid transfer.

MATERIALS AND METHODS

Three liters of porcine blood were obtained from a local processing facility after attaining proper approvals. The blood was heparinized to a concentration of 10 units/mL and strained using cheesecloth to remove large debris and clots. The blood was circulated through a 6-ft tubing loop that passed through a Medtronic Affinity Cardiotomy Venous Reservoir (Medtronic, Minneapolis, MN) with a 120-μm filter until it reached room temperature. A pre-calculated amount of soybean oil was added as the blood circulated to reach an optimum triglyceride level similar to that of human blood with hypertriglyceridemia (>150 mg/dL). The blood was circulated for 10 minutes after introduction of the soybean oil. The hematocrit of the blood was determined to be 12%; this served as an approximation for shed blood returned from the chest.

One liter of blood was transferred for testing into a Fresenius ATR120 Autotransfusion Reservoir (Fresenius Kabi, Bad Homburg, Germany) with a 120-μm filter. Samples were collected at regular intervals of 0, 15, 30, 45, and 60 minutes in the following manner.

A syringe was placed on a stopcock at the outlet of the reservoir. One hundred milliliters of waste was discarded, and two 4-mL samples were collected and allocated into serum separator tubes. An additional 92 mL was drained for a total volume of 200 mL drained at each time interval. Two samples collected at each of the five intervals yielded a total of 10 samples. This trial was conducted three times to reach a total of 30 samples. The samples were tested for total triglyceride content with the IDEXX VetTest 8008 Chemistry Analyzer (IDEXX Laboratories, Westbrook, ME).

Results were analyzed with SPSS 12.0 (SPSS, Chicago, IL) using repeated-measures ANOVA, and a post hoc Bonferroni test was used. The α value was 0.05.

RESULTS

The mean and SD for total triglyceride content in milligrams per decaliter are shown in Table 1. The 0-minute group was used as a baseline, and this was compared with the other four time groups with repeated-measures analysis. There was a significant effect for time (Wilkes lambda = .013, $F = 39.437$, $p < .05$). Post hoc analysis using Bonferroni showed significant differences ($p < .05$) between 0 and 45, 0 and 60, 15 and 45, 15 and 60, 30 and 45, and 30 and 60 minutes. The reduction rate of triglycerides was calculated to be .29 mg/dL/min.

DISCUSSION

With the prevalence of high-risk patients and more reoperations, longer bypass runs are necessary in many cases for surgical procedures. These longer CPB times are associated with greater neurologic deficits caused by lipid microemboli (13). Hypothermia is used for many complex procedures, but hypothermia with cardiotomy suction has been identified as causing 10 times more SCADs than normothermic CPB or CPB without cardiotomy suction. Liquid fat emboli are responsible for the increased clogging rate of pericardial suction blood through cerebral capillaries as shown by Appelblad and Engstrom (14).

Our hypothesis was similar to that of a previous study (12) in that we thought fat content could be reduced in cardiotomy suction blood by gravity separation. They performed an experiment testing the separation of soybean oil and human fat in blood at several temperatures. Soybean oil is used as a comparative reference to human fats in several studies because of similarities in fatty acid distribution

Table 1. Triglyceride levels ($n = 6$ trials).

Time (minutes)	Triglyceride Level (mg/dL)
0	75.67 ± 2.80
15	75.33 ± 3.08
30	74.33 ± 5.13
45	65.67 ± 6.95
60	58.50 ± 2.88

Significant differences ($p < .05$) in triglycerides (mg/dL) were found between 0 and 45, 0 and 60, 15 and 45, 15 and 60, 30 and 45, and 30 and 60 minutes.

and structures (15). The results proved to be very promising. At 37°C, >90% of soybean oil and 65% of human fat separated from the blood; human fat consistently separated in higher percentages at 20°C (71%) and 10°C (78%). No less than 86% of the soybean oil separated from the blood at any temperature. Additionally, the authors were able to determine the extent of surface adhesion of the fat to the container wall in their experiment, and they found it to be a very significant source of fat elimination (12), which greatly added to the effectiveness of the technique.

Our experiment intended to recreate this scenario on a larger scale. The results showed significant differences in fat concentration ($p < .05$) between baseline and 45 minutes, baseline and 60 minutes, 15 and 45 minutes, 15 and 60 minutes, 30 and 45 minutes, and 30 and 60 minutes. This shows that fat effectively separated from the blood at 45 and 60 minutes.

Although gravity separation does occur over time, it seems to be more effective after 30 minutes. With most routine coronary artery bypass graft (CABG) surgeries, this technique does not seem to be feasible because of reduced amounts of cardiotomy suction blood; however, complex surgeries such as aortic procedures, multiple valve procedures, re-operations, transplantations, and combination procedures could be a better arena to attempt this technique. Other areas of potential use for this technique could be orthopedics cases and the re-infusion of postoperative mediastinal shed blood. This could salvage valuable red blood cells and proteins, possibly requiring less blood product transfusions in these scenarios.

We were able to calculate the rate of fat separation from blood to .29 mg/dL/min. Using this, we could predict the approximate amount of time needed to reduce the fat concentration to a range that is more clinically acceptable before re-infusion of the blood.

Conducting this experiment on a clinical scale using human fat and human blood is the next step in evaluation of this technique; one reason is because soybean oil has been noted to behave differently than human fat in similar experiments (12). The level of triglycerides reached in this study was below the desired level. This could be because of the inability of the testing equipment to recognize the soybean oil or binding of proteins in the porcine blood to the oil. Samples should also be examined with a higher hemoglobin level to determine whether the lipid separation is affected by hemoglobin concentration. The authors concluded that the sample's low hematocrit was caused by dilution related to the collection process and clotting that may have occurred before heparinization of the porcine blood. Another factor to consider is stagnation of the blood in the reservoir. Regardless of heparinization, stagnant blood will eventually clot. Unfortunately, this seems to be a necessary element of this technique with larger volumes of blood. This would beckon a filtering mechanism; however, cost of materials may now become an issue, negating a major advantage.

In conclusion, we were able to significantly reduce the amount of soybean oil in the blood by allowing it to settle, undisturbed, for >30 minutes. At 45 minutes, a 13% reduction was noted, and 23% was eliminated at 1 hour. The re-emergence of this technique could open new doors to the elimination of fat from cardiotomy suction blood without adding unnecessary high costs to the patient.

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