

**Citrate Anticoagulant and Apheresis
Perspective of Plasma Protein Therapeutics Industry**
November 29, 2012

Erratum: March 19, 2013. Reference error in paper; paper corrected.

Background

Following the meeting of the Blood Commission on November 16, 2011, where the Amrein et al. study of plateletpheresis donors that reported bone mineral density (BMD) losses at one of three skeletal sites was associated with donating platelets¹ was discussed, the Plasma Protein Therapeutics Association (PPTA) was asked to respond to the suggestion that Source Plasma donors, with their high-frequency, high-volume donations, were at risk of BMD loss. The industry has evaluated the Amrein et al. study, conducted extensive literature surveys to see if similar findings had been published, and assessed risk to plasma donors looking at the comparison of the exposures to citrate that plasma and platelet donors would receive during the apheresis collection process. The result of this analysis is presented in this paper to address the Blood Commission's concerns.

Calcium – body distribution

Calcium is the most abundant mineral in the human body. The average adult body contains about 1000 grams of calcium. Ninety-nine (99) % of the calcium is in the skeleton in the form of calcium phosphate salts. The rest is in the extracellular fluid (ECF) but only about one tenth of one percent of this amount is present in the blood (normal blood calcium concentration, 10 mg/dL). Calcium is distributed in the blood as follows:

- About 40% bound to proteins (mainly albumin).
- About 13% bound to naturally present small anions (ligands) such as phosphate, citrate, and lactate.
- About 47% ionized form. (*Ionized* calcium is calcium that is freely flowing in the blood and not attached to proteins; it is also called free calcium.)²

These proportions differ with changes in pH and total concentration of calcium, protein, and ligands.^{2,3}

Apheresis and calcium

To collect blood components by apheresis, it is necessary to anticoagulate the blood drawn from the donor to prevent clotting within the apheresis device. Citrate effectively chelates divalent cations, such as calcium, to inhibit immediately (and transiently) the coagulation cascade. Since the citrate binds with a portion of the *ionized calcium*, the activation of the calcium-dependent coagulation factors is effectively blocked.²

Acute, short-term changes in ionized calcium levels during the apheresis process are well known.^{4,5,6,7} When the concentration of ionized calcium decreases, it results in many of the acute clinical signs and symptoms of "citrate toxicity," such as perioral tingling and paresthesias, chills, nausea, twitching, and tremors. If citrate concentrations rise excessively consequent to the rapid administration of large amounts of citrate, the ionized calcium may decrease significantly and more severe symptoms, such as carpopedal spasm, seizures, tetany, and cardiac arrhythmias, may occur. These side effects are well known, and prompt attention to mild symptoms typically requires only pausing or stopping the procedure to reduce the reinfusion rate. This helps prevent more severe citrate reactions.

Sequela of citrate toxicity are dependent on the rate of citrate administration, the duration of the infusion, the dilution of citrate in the ECF, redistribution, rate of metabolism, and the rate at which citrate is excreted. The liver, kidney, and skeletal muscle are responsible for most of the metabolism and excretion of citrate. Experimental studies showed a rapid initial citrate clearance of 50% over the first 30 minutes followed by a more gradual clearance of the remaining 50% over the next two and half hours.³ The citrate level in serum and urine typically returns to baseline within 4 hours after the infusion has stopped.² In the urine, the acute citrate load produces cation excretion including calcium, magnesium, sodium, and potassium.

In addition, when plasma-ionized calcium decreases, the parathyroid glands sense the change and secrete parathyroid hormone (PTH) immediately. The PTH increases quickly within 5 to 15 minutes after citrate infusion has been initiated.² A study on plateletpheresis showed that the intact PTH rises quickly, then levels off or slightly decreases during the remainder of the procedure, despite progressive decreases in calcium.² PTH raises calcium levels by: a) releasing calcium from the large reservoir contained in the bones, b) enhancing active reabsorption of calcium in the kidney and c) enhancing the absorption of calcium in the small intestine by increasing the production of activated vitamin D.⁸

Factors affecting the amount of citrate infused to apheresis donors

Equipment used: The total citrate infused per procedure depends on the adjusted “citrate infusion rate.” Calculation of the citrate infusion rate in terms of the weight of the donor allows compensating for variations in the body mass of different donors. The citrate infusion rate normalizes all donors by dividing the infusion rate per minute by the donor's body weight in kilograms. It is expressed in terms of *mg of citrate administered per kilogram body weight per minute (mg/kg/min)*.

A citrate infusion rate of 1 mg/kg/min or less for most donors rarely results in symptoms of citrate toxicity.⁹ A citrate infusion rate of 1.7 mg/kg/min or greater, however, may be associated with risk of moderate to severe acute citrate reactions. The higher citrate infusion rate may exceed the rate at which citrate can be metabolized causing a decrease in ionized calcium levels in the blood and the appearance of hypocalcemic symptoms.⁹

However, the size of the donor affects the rate of citrate accumulation. As a consequence, at similar citrate infusion rate, smaller donors may experience acute symptoms of citrate toxicity at a greater frequency than do larger donors, due to the fact that there is less ECF for dilution and a lesser mass of tissue to metabolize the compound. The goal of citrate anticoagulation is to infuse sufficient citrate to prevent blood clotting in the extracorporeal circuit but not so much as to lead to significant citrate reaction symptoms in the donor.

Type of apheresis product collected: Depending on the type of procedure being performed, the amount of citrate returned to the donor varies widely. For example, during *plateletpheresis* procedures, the red cells and nearly all plasma are returned to the donor. Therefore, most of the citrate that is added to the blood during the procedure goes back to the donor. However, a significantly lower amount of citrate is returned to the donor during *plasmapheresis* (e.g., Source Plasma) since most of the plasma is collected (not returned to the donor).

Frequency of donations in Austria: Apheresis donors are allowed to donate at different intervals depending on the blood component to be collected:

- Plateletpheresis – up to 26 times a year
- Plasmapheresis – up to 50 times a year¹⁰

Type of anticoagulant used: For the collection of products for transfusion (platelets, red cells, plasma, etc.) the Anticoagulant Citrate Dextrose solution (ACD-A) is typically used. However, for collection of Source Plasma, a 4% sodium citrate anticoagulant solution is used. The total citrate ion in the anticoagulant solution may be calculated as follows:

A 4% Sodium Citrate solution contains 4 grams of trisodium citrate dihydrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$) per 100 mL of solution (40 mg/mL). The molecular weight of the compound is 294, and the molecular weight of citrate ion is 189. Therefore, each mL of sodium citrate anticoagulant contains $40 \text{ mg/mL} \times 189/294 = \underline{25.7 \text{ mg of citrate ion}}$.

Other variables: There are several parameters that affect the total citrate infused to the donors during plasmapheresis procedures (e.g., product volume, donor hematocrit, donor plasma volume).

Citrate Exposure and Ionized Calcium Loss

The *AC delivered* to the donor as shown in the graphs provided by Fenwal (Fig. 1 and Fig. 2) indicates total volume of anticoagulant infused to the donor during a plasmapheresis procedure.¹¹ Volume of anticoagulant returned to the donor is a function of the donor's hematocrit. The highest citrate exposure as seen on Fig. 1 is when 880 mL is collected from very high hematocrit donors (54%). *AC delivered* is not sensitive to donor weight or flow rates. Hematocrit and donation volume are determining factors for donor citrate exposure.

If the donor's hematocrit is 40%, and 880 mL of anticoagulated plasma is collected, the estimated total anticoagulant volume infused to the donor is about 58 mL (Fig. 1). Thus, the 80 kg donor would be exposed to 1491 mg citrate. The plasmapheresis process usually takes from 1 to 1 ½ hours. For this donor, then, the exposure would be $1491 \text{ mg}/80 \text{ kg}/60 \text{ min}$ or 0.31 mg/kg/min , the citrate infusion rate. From Fig. 2, it can be seen that, if only 650 mL plasma is collected, the citrate exposure will be correspondingly lower with the same hematocrit. For example, the 80 kg donor shown in Fig. 2 with a hematocrit of 40% would have approximately 45 mL of anticoagulant infused for a citrate infusion rate of 0.24 mg/kg/min .

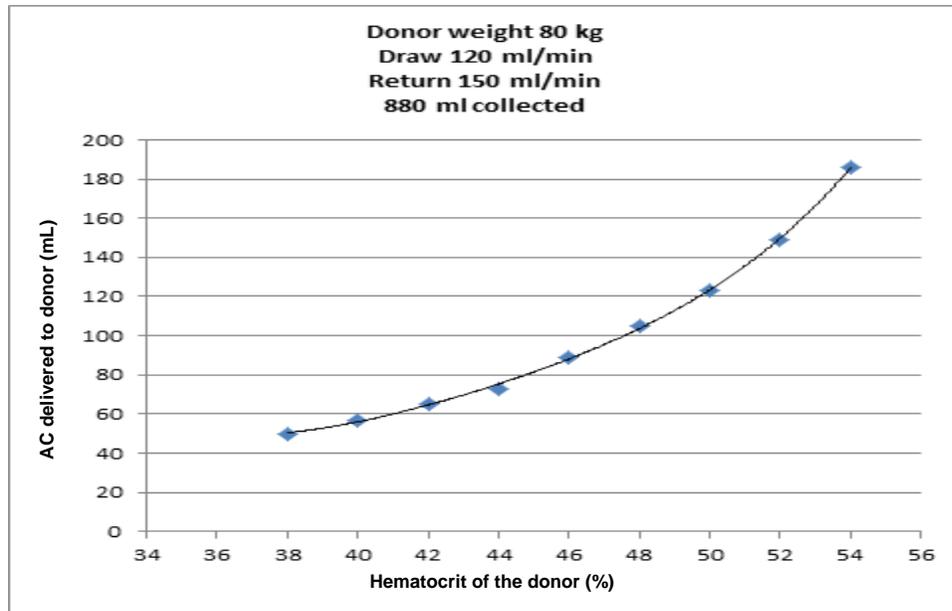


Fig. 1 (Fenwal)¹¹

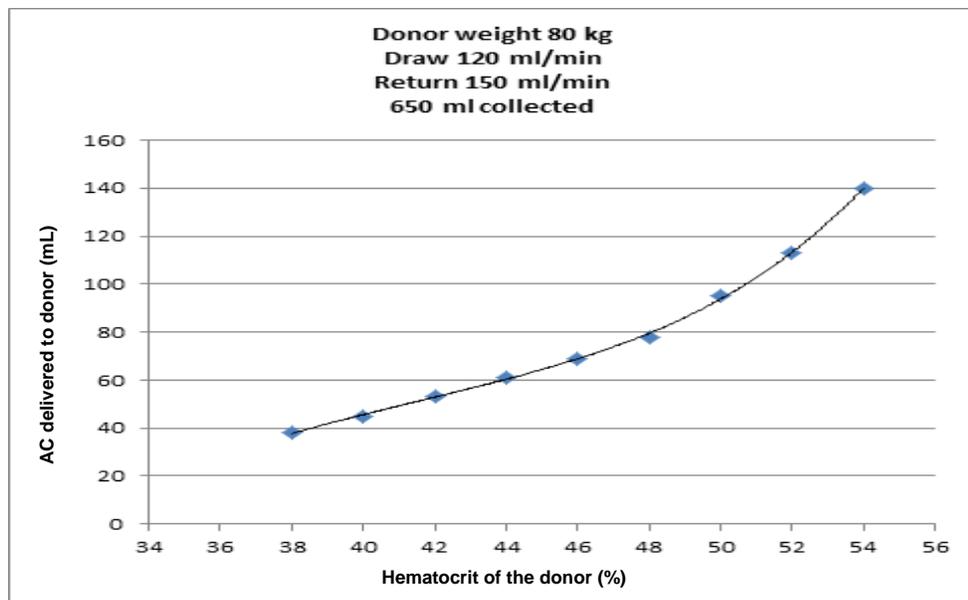


Fig. 2 (Fenwal)¹¹

During plateletpheresis, the amount of citrate the donor would receive is roughly equal to that added to the blood as it enters the collection system since almost all the plasma is returned to the donor during the apheresis procedure. Only a small amount of plasma is retained during the processing. In contrast, the plasma donor will receive substantially less citrate since most of the added citrate remains in the collected plasma.

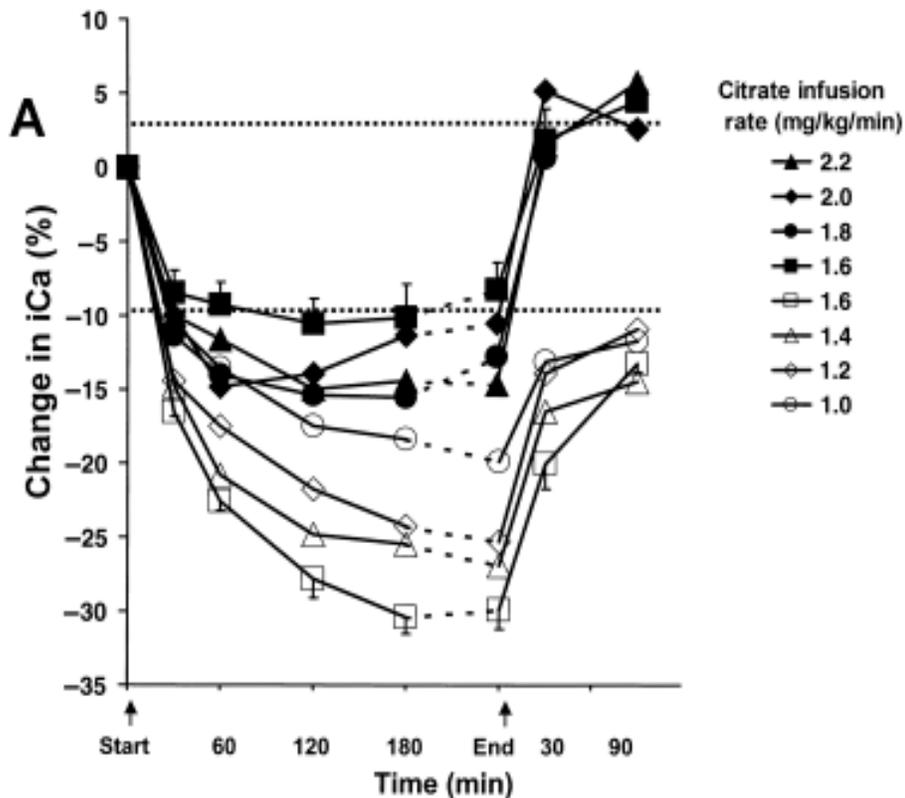


Fig. 3. Changes in serum ionized calcium concentrations during leukapheresis. Dotted lines indicate upper and lower limits of the reference range. Open symbols (□, △, ◇, and ○) represent procedures without prophylactic Ca, solid symbols (■, ▲, ◆, and ●) those with Ca. Levels of ionized calcium were markedly lower in procedures performed without prophylactic Ca and remained significantly below the reference range 90 minutes after completion of apheresis. (Bolan)¹²

While we have based our illustrative calculations of citrate exposure above on data provided by Fenwal, similar results would be expected according to data provided by Haemonetics, the other major apheresis equipment supplier.¹³ Data supplied by Haemonetics indicate that citrate exposure during plateletpheresis is reported to be approximately 8 times higher than that received by plasmapheresis.¹³ Thus, for donors with equal hematocrit, the plasma donor would have to give 8 times the number of donations to receive the same cumulative citrate exposure as the platelet donor.

For plateletpheresis or peripheral blood progenitor cell collection, the citrate infusion rate appears to be 1.5 mg/kg/min, which is about 5 times the citrate infusion rate used in plasmapheresis.^{4,6,7,12} Ionized calcium loss appears to be dose dependent, as shown by Bolan in the study of various citrate infusion rates with and without administration of calcium.¹² With the citrate infusion rate of 1.6 mg/kg/min at 60 minutes, there was a drop in ionized calcium of about 22.5% versus a drop of about 13.0% with a rate of 1.0 as shown in Fig. 3. For a citrate infusion rate of 1.5 mg/kg/min, the ionized calcium loss is calculated to be about 20.92% extrapolating from Fig. 3. With the citrate infusion rate of 0.31 mg/kg/min used for plasmapheresis, a decrease of about 2.1% in the ionized calcium would be expected assuming linearity of response. Thus, with the citrate infusion rate for plasmapheresis, ionized calcium loss is about 10% of that seen with the high exposure level employed for plateletpheresis. If the effects were to be cumulative, there would have to be 10.0 times as many plasma donations to have the same cumulative level of ionized calcium loss. This figure is comparable to the 8-fold differential citrate exposure for the two processes as indicated in data from Haemonetics. Moreover, data provided by Haemonetics for 30 plasma donors indicates an average citrate infusion of about 23 mL for donors with a range of hematocrits and weights.¹³ Thus, ionized calcium loss on average would be lower than the figure used in our illustration.

In Austria, donors are allowed to give 26 platelet donations and 50 plasma donations per year. Thus, with a ratio of 1.92 plasma/platelet donations allowed, the donor giving the maximum number of plasmapheresis donations would still have 80% less potential ionized calcium losses than the platelet donor giving at the maximum frequency. Few donors, however, give at the maximum number of times. It is also questionable that, with the loss in ionized calcium of only 10% seen with plateletpheresis, there would be the same effect on bone demineralization, given that the ionized calcium decrease is only short term.¹ In addition, it is expected that the transitory rise in PTH, the hormone affecting bone loss, reported by Amrein et al., would be correspondingly less, thus further minimizing possible bone calcium metabolism.¹ Amrein et al. reported a small and marginally statistically significant BMD difference between plateletpheresis donors and controls.¹ In addition, the loss was only significant at one of three skeletal measurement sites. If the effect of repeated citrate exposure is associated with significant BMD loss, it is surprising that the difference in the femoral neck was the smallest since this site frequently serves as the reference skeletal site for epidemiological studies in defining osteoporosis.¹⁴

Summary

The acute effects of citrate are recognized and are rapidly reversible because it is metabolized within minutes in the liver, kidneys, and muscles and other compensatory mechanisms, such as the release of PTH that mobilizes calcium from the reservoir in the bones, increases reabsorption of calcium in the kidney and enhances absorption of calcium in the small intestine.^{2,9} While there is clear evidence that short term calcium metabolism is affected by exposure to citrate during apheresis procedures, evidence that there is a resulting clinically significant change in bone metabolism due to the increased renal excretion of calcium citrate is lacking. Moreover, in the absence of longitudinal studies, the long-term effects of repeated apheresis procedures on calcium balance and BMD are not known.

Amrein et al.'s cross sectional study of plateletpheresis donors has, however, reported a small but statistically significant association with lumbar BMD.¹ The authors indicate that further studies are needed to delineate the effects of acute changes in serum ionized calcium, PTH, and blood pH on bone cell activity under in-vitro and in-vivo conditions before one can better interpret such data. Of note is the statement that the authors were unable to find a significant relationship between donation frequency and lumbar spine Z-scores. As they state, there might not have been sufficient power for this type analysis, but a dose response relationship with exposure would be expected if the reported relationship is meaningful.

Some of the limitations of the Amrein et al. publication are:

- Heterogeneity of the study population: Donation type (combination of plateletpheresis/plasmapheresis or plateletpheresis only), donation frequency (variable 16 – 633), and apheresis devices (three different types used).
- No information regarding type of anticoagulant used or the citrate infusion rate used.
- No information regarding when was the last donation (e.g., the paper indicates “donors had an average of 85 apheresis procedures in the past”).
- Only one of the three measurement sites (lumbar) for BMD showed results that were statistically significant. However, the other two sites (total hip and femoral neck) were not affected.

Conclusion

While the new report suggests that plateletpheresis donors might have a higher prevalence of lumbar BMD loss, the extension of this to plasmapheresis donors is hypothetical based on the limited findings of the Amrein et al. study.¹ In fact, an earlier report that examined BMD changes in platelet donors who had given more than 50 donations over a 10 year period found no significant changes in age-adjusted BMD at the lumbar spine, hip and radius sites when compared to individuals of the same gender and race who were whole blood donors.¹⁵ These authors conclude that “frequent apheresis donations do not appear to have long-term effects on baseline laboratory levels or [BMD] measurements.”

Citrate exposure for plasmapheresed donors is only about 1/5th to 1/8th that of the exposure that platelet donors experience. Evidence shows that at this level the acute and transitory changes in ionized calcium would be minimal. In all studies, the biological parameters are normalized after 24 hours, so the true significance of the reported changes for the Austrian platelet donors is unclear. In addition, the plateletpheresed donor would be exposed to from 2 ½ to 4 times the cumulative citrate as the plasmapheresed donor if each gives the maximum number of allowable donations. In terms of cumulative ionized calcium loss the platelet donor would have greater than 5 times the loss as the plasma donor. Only a well-designed long term longitudinal study would be able to draw any meaningful conclusions about the impact of citrate exposure on BMD loss. However, with the anticipated small loss in ionized calcium during the plasma collection process, it is doubtful whether significant BMD changes would be observed.

Evidence exists that remedial actions (e.g., calcium supplementation) can mitigate the acute calcium loss from citrate exposure during apheresis.^{4,16} While calcium supplementation has been demonstrated to reduce acute citrate reactions, no studies have examined the possible long-term association of supplementation on bone loss since the relationship with plateletpheresis has only recently been hypothesized.

Other than the Amrein et al. report, conducted on platelet donors, suggesting a concern with BMD loss in donors from plasmapheresis, there is no other evidence supporting a concern with bone demineralization. With the much lower citrate exposure during plasmapheresis it appears to be premature to suggest that these donors are at additional risk of bone demineralization.

Donors have safely donated Source Plasma for the manufacture of plasma protein therapies for decades. The health of donors is assessed prior to the first donation by acquiring health histories, and performing limited physical examinations, measurements of donor health parameters (e.g., donor weight, blood pressure, pulse, temperature, tests for hematocrit, protein, infectious diseases). These measures are repeated on a regular time frame. The plasma industry is dedicated to safeguarding the health of people who are committed to donating plasma for manufacturing lifesaving products. The health of donors is an industry priority, and plasma collectors in concert with PPTA will continue to safeguard the health of donors.

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