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Effect of Plasma Unit Weight and Donor Sex on Post-Donation Citrate Level: An Experimental Study on Plasmapheresis Donors

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Keywords
Plasmapheresis · ACD-A · Citrate metabolism · Personalized plasmapheresis · Sex · Transfusion medicine

Abstract

Introduction: Plasmapheresis donation is considered safe and well tolerated, although long-term effects need to be clarified. The volumes of anticoagulant (ACD-A) used are variable and depend primarily on hematocrit (HCT), total blood processed, amount of plasma collected, and donor characteristics. To elucidate the effect of the plasma unit weight setting on plasmapheresis efficiency and ACD-A distribution, we enrolled male donors undergoing a controlled apheresis process donating 700 g and 720 g in two different sessions. In parallel, we investigated a possible effect of sex, recruiting women donating 700 g of plasma. ***Methods:*** The study was conducted on men donating 720 g and (12 months later) 700 g of plasma, and on women donating 700 g of plasma. The main outcomes were pre-/post-donation delta (Δ) citrate concentration in donor plasma and ACD-A reinfused to the donor. Information concerning the annual check-up and the procedure was also collected. Intergroup comparisons (men donating 720 g vs. men donating 700 g and men vs. women both donating 700 g) and intragroup associations with donor and procedural characteristics were reported. ***Results:*** With the procedure set at 720 g, the machine processed around 44 mL more whole blood to collect 20 g more plasma, and 720 g donors received around 12 mL more anticoagulant than 700 g donors. Accordingly, Δ citrate concentration was 1.5 times higher (12 μM), with a greater variability observed for 720 g donations. Citrate concentration in the plasma unit was lower in the 720 g group, although not significantly. Comparing outcomes between women and men donating 700 g, we observed higher (and highly variable) Δ citrate and reinfused ACD-A in women, accompanied by lower anticoagulant levels in the unit. Increased Δ citrate is inversely associated with HCT and age in men and with HCT and triglycerides in women. Reinfused ACD-A correlates with HCT in women but not in men. ***Conclusion:*** Unit weight setting and sex influence an ACD-A shift from the estimated values toward an increased reinfusion to donor. In parallel, we observed an impact of age and sex on post-donation citrate metabolism. Altogether, these elements should be taken into account for the development of tailored approaches aimed at maintaining similar safety profiles for all donors using different plasmapheresis settings.

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Introduction

Because donor plasma is the source of plasma-derived medicinal products, collection of this substance must be maintained in both normal and exceptional circumstances, such as those experienced during the COVID-19 pandemic [1, 2], ensuring at the same time that donor safety is preserved. In current practice, plasma collection is often performed using plasmapheresis, an automated collection system by which plasma is collected using a cell separator, while the remaining components are returned to the donor. An extracorporeal circuit is used to remove plasma with addition of an anticoagulant (usually citrate-dextrose solution A, ACD-A) in order to prevent clotting during blood processing. International and national guidelines regulate plasmapheresis donation throughout Europe and the world, and the main differences relate to the frequency of donation and the volume of plasma collected [3, 4]. Depending on the amount of blood processed and plasma collected, variable volumes of citrate-containing anticoagulant are delivered to the collected unit or reinfused to the donor. According to the manufacturer's instructions, which are based on specific machine parameters and settings, it is possible to estimate the ACD-A volume in the final product and consequently, the volume of ACD-A returned to the donor [5]. However, the estimated ACD-A can differ significantly from the real amount in the final unit [6], due to donor and procedure characteristics that are not taken into account by the machine algorithms [7].

Although plasmapheresis is generally well tolerated, citrate-mediated hypocalcemia due to ACD-A reinfusion can cause short-term moderate acute adverse effects (AEs; such as muscle cramps or nausea) [6, 8–11]. Literature studies have hypothesized a possible correlation between citrate-containing anticoagulant exposure and long-term effects in frequent donors (and particularly in women), such as altered bone resorption and deposition [12, 13]. Given the diversity among apheresis protocols performed worldwide and the different responses to citrate exposure due to donor variability [12], it is important to assess the exact distribution of the anticoagulant between the donor and the plasma unit.

In a recent paper comparing two blood donor centers, with apparently identical plasmapheresis protocols and machines (PCS2; Haemonetics Corp., Rosemont, IL, USA) but different plasma unit weight settings (700 g vs. 720 g), we observed a diminished efficiency of the procedure when plasma collection was extended over 700 g [14]. Our observational data suggested that a shift in the setting from 700 g to 720 g was associated with a small increase in net plasma in the final unit (4 mL) but implied an increase of over 100 mL in total blood processed and a higher amount of ACD-A in plasma collected. Even though the procedures were apparently conducted under similar conditions, the two groups of donors were not comparable, and the machines were located in two different clinics and managed by different professionals. Literature studies agree on the importance of proper equipment and disposables assembly procedures, and the use of personnel experienced in venipuncture, in guaranteeing efficient collection [15]. We cannot therefore exclude the possibility that the differences observed in the efficiency of the process in two donor centers were not merely due to different weight settings.

Thus, we conducted an experimental study on a single center, in which the same subjects underwent a controlled plasmapheresis process using the same machine (PCS2), and with the final volume set first at 720 g and in the following session at 700 g. Since apheresis at 720 g is rarely performed in women in our donor centers because of local health authority policy, this comparison included only men.

Nevertheless, our previous observational study [14] also comprised data from a few women. It is therefore possible to exclude (or confirm) a potential sex effect on the efficiency of plasmapheresis and citrate distribution between unit and donor, and we also recruited female subjects with the plasma donation level set at 700 g.

For both aims, the estimated amount of ACD-A reinfused to the donor and the pre-/post-donation delta of plasma citrate concentration in donor blood were considered as primary variables for comparison. Other donor and procedural variables were then measured in terms of the two outcomes within each group. The weight settings (700 g and 720 g) were selected since they fell in the range described by the Italian law [3] which states that the minimum amount of plasma collected must be 600 mL net of the anticoagulant. Setting less than 700 g total weight would have led to discarding the large majority of the collected units. Conversely, in our centers, we avoid using weights higher than 720 g to preserve donor safety.

Materials and Methods

Study Design and Participants

Data collected for this study comprise a total number of 61 procedures on male donors with machines set for the collection of 700 g of plasma and 61 for the collection of 720 g, performed approximately 6 months before the 700 g collection, as well as 72 procedures on female donors (with machines set at 700 g). Details on the data collected are provided in online supplementary Table SI1 in the online supplementary information (for all online suppl. material, see www.karger.com/doi/10.1159/000529394).

The main outcomes were:

1. Difference between the measured pre-apheresis and post-apheresis plasma citrate concentration in donors (pre-/post-donation Δ citrate).
2. Measured ACD-A volume infused to the donor at the end of the procedure.

Pre-/post-donation Δ citrate was assessed as the intraindividual difference between the citrate plasma concentration measured before plasmapheresis and after plasmapheresis (blood sample obtained immediately after the end of the procedure). The measured ACD-A volume infused to the donor was calculated by subtracting the citrate measured in the plasma unit from the total ACD-A used by the machine.

Plasma collection was performed at the selected center (Casa del Dono di Reggio Emilia, Reggio Emilia, Italy), in accordance with the Italian Ministerial Decree of November 2, 2015 [3], using the Plasma Collection System (PCS2, Haemonetics Corp., Rosemont, IL, USA), following the manufacturer's instructions [16]. The PCS2 machine and the anticoagulant used (ACD-A; Haemonetics Corp., Rosemont, IL, USA) were the same as in our previously published communication [14]. All donors were recruited between February 2022 and March 2021 (Fig. 1a) and signed an informed consent form before being enrolled in the study.

Italian Eligibility Criteria for Plasmapheresis Donation

- Age between 18 and 65 years old (60 years for intensive plasmapheresis program).
- Weight not less than 50 kg.
- Systolic blood pressure ≤180 mm Hg.
- Diastolic blood pressure ≤100 mm Hg.
- Hearth rate between 50 and 100 beats/min.
- Hb ≥13.5 g/dL for male donors.
- Hb ≥12.5 g/dL for female donors.
- Total protein ≥6 g/dL and normal electrophoretic picture.

The donor placed in an intensive plasmapheresis program must undergo periodic checks at least every 6 months and be carefully evaluated by the medical expert in transfusion medicine regarding possible significant decreases in the proteinemia values and abnormalities of the electrophoretic picture [3].

Procedure Parameters

Information concerning the procedure (such as blood volume processed, total amount of ACD-A used, plasma unit weight, number of cycles of the procedure, collection time) was collected from the PCS2 machine display after each donation by the trained personnel at the donor center. Plasma collection was performed in all cases using the same needle gauge (18 G), protocol, speed, and number of cycles (n = 4, for both males and females). A total volume of 250 mL of saline solution was administered during the procedure and an additional 250 mL on completion. In our donor centers, indeed, saline compensation is often necessary in routine practice: therefore, we decided to compensate all the recruited donors to minimize the differences between procedures. All donors were monitored during and after plasma apheresis (at least 15 min of observation were assured to wait for the post-apheresis blood sample collection) for acute adverse events, including vasovagal reactions, nausea, tingling in the lips, malaise in general. Hematomas were not monitored in the post-donation period. The plasmapheresis software used is specific for the PCS2 machine. The draw and return flow was set at 80 mL/min as default: the machine automatically modulates the flow at the beginning and end of the procedure. ACD-A/whole blood ratio was set at 1:12. The centrifuge speed was set at 7,000 rpm, as provided by the manufacturer. The plasmapheresis machine used in this study (PCS2) provides information on the product volume, such as total volume of ACD-A used during the procedure. The amount of anticoagulant (ACD-A) used during a plasmapheresis procedure is measured by taking into account the revolution of the pump based on the ACD-A:blood ratio (1:12).

Sample and Data Collection

Immediately before and after plasmapheresis donation, 7 mL aliquots of peripheral blood were collected from each donor in heparin-containing tubes to measure the following parameters: plasma citrate, plasma density, and total proteins. The post-apheresis blood sample was collected from the same vein after disconnection of the apheresis kit. Blood samples were collected 15 min after disconnection, in order to allow the distribution of reinfused anticoagulant and saline in the systemic circulation. Briefly, whole blood was centrifuged at 2,500 *g* for 10 min, and plasma supernatants were split into further tubes to perform additional assessments. Plasma samples from the collected units were recovered from the connection tube without affecting bag integrity and safety, as described [6], and were prepared for use in citrate and total protein quantification and for plasma density assessment.

For both donor and unit plasma samples, a 2 mL aliquot was collected in an empty tube and used to measure total proteins. Total proteins were quantified on an AEROSET c8000 system (Abbott Diagnostic, Chicago, IL, USA) following the manufacturer's instructions by the Chemical-Clinical Analyses (LACCE) Hospital Laboratory. The remaining plasma was used to assess plasma density (see plasma density section) and in part stored in cryovials at −80°C for citrate assessment. The study scheme for sample collection and analyses is shown in Figure 1b.

Donor Characteristics

Donor characteristics were measured as routinely performed before each donation. Blood count and hematocrit (HCT) were quantified on 1 mL of donor whole blood using a CELL-DYN Ruby Hematology Analyzer (Abbott Laboratories, Chicago, IL, USA). Total cholesterol, triglycerides, and glucose were quantified on an AEROSET c8000 system (Abbott Diagnostic) following the manufacturer's instructions. The medical personnel at the donor center collected information on sex, age, weight, height, blood group, and blood pressure.

Plasma Citrate Quantification

Since ACD-A is a citrate-containing anticoagulant, we measured plasma citrate changes to monitor its distribution between donors and plasma units. Plasma citrate collected from donor peripheral blood before and after plasmapheresis and from collected plasma units was measured by means of a colorimetric assay (MAK057 Citrate Assay Kit, Sigma-Aldrich, USA). Plasma aliquots were first heated at 65°C for 60 min to allow plasma proteins to degrade. Next, aliquots were centrifuged at 21,000 *g* for 30 min at 37°C to remove debris [6]. Plasmapheresis samples were appropriately diluted (1:100) to fall within the kit's detection range. Plasma samples falling outside the detection range were excluded from further analyses.

Plasma Density

Pre- and post-apheresis donor plasma density, as well as plasma unit density, were assessed on a 2 mL sample using a DMA 4500 M Chemicals Viscosimeter (Anton-Paar GmbH) as previously described [6].

Estimated Parameters

The ACD-A volume in the plasma unit and in the donors (donor blood volume) was calculated as previously described (measured ACD-A) [6] and as described in manufacturer's manual (estimated ACD-A) [16]. Citrate concentration was estimated from the ACD volume considering the concentration of citrate in the ACD-A solution used. Post-apheresis citrate was normalized for the pre-/post-apheresis plasma density ratio in order to exclude

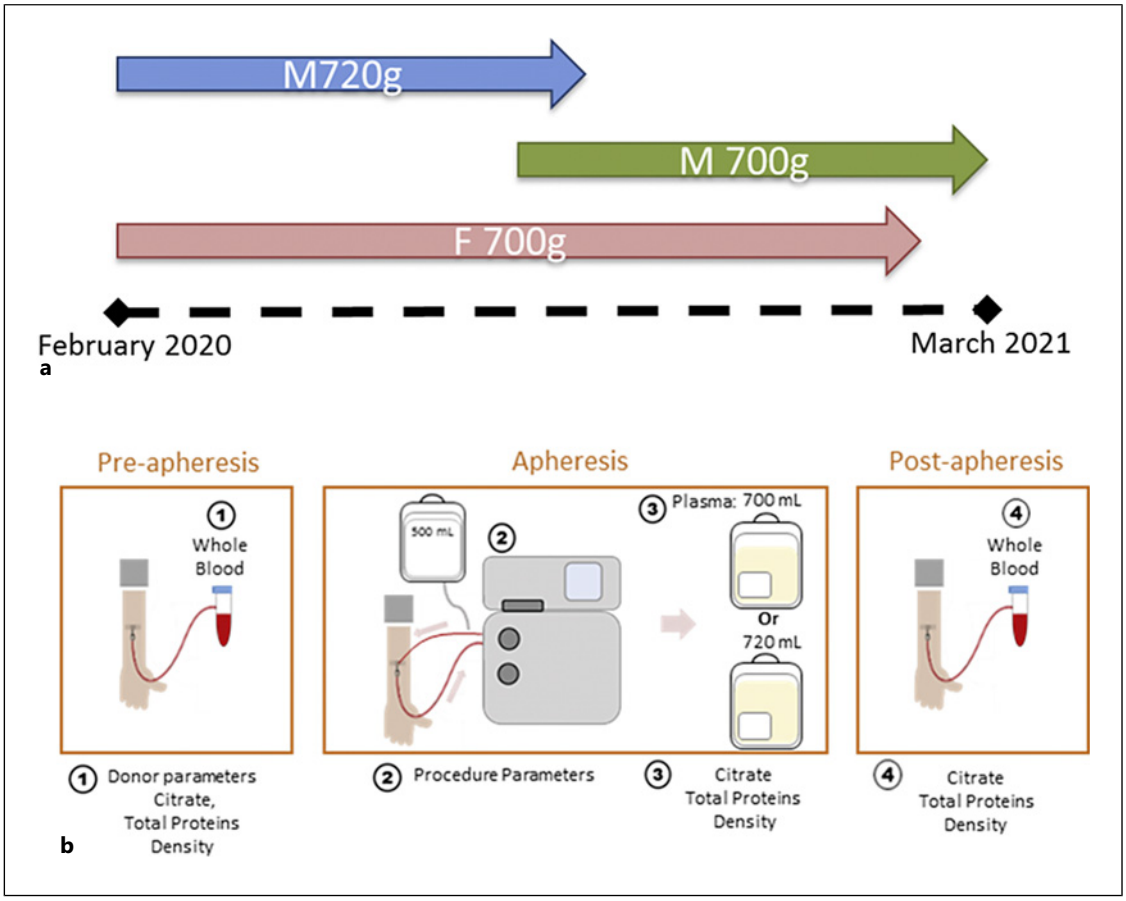

Fig. 1. a Study timeline. **b** Scheme of the samples and data collection. M 720 = males donating 720 g of plasma; M 700 = males donating 700 g of plasma; F 700 = females donating 700 g of plasma.

the impact of saline solution infusion in citrate measurement. The pre-/post-donation Δ citrate concentration was calculated by subtracting the pre-apheresis citrate values from the normalized post-apheresis citrate values.

Statistics and Data Analysis

Descriptive analyses of donor characteristics and related procedures were performed by group (male donors at 700 g and 720 g weight setting and female donors at 700 g weight setting). Differences between paired data for male donors and differences between male versus female donors at 700 g weight setting with a valid value for pre-/post-donation Δ citrate in donor plasma were compared using Student's *t* test. Linear regression models were used to evaluate the associations between donor characteristics and plasmapheresis procedures and the two outcomes, namely, pre-/post-donation Δ citrate concentration in plasma and ACD-A volume reinfused.

Comparisons on paired data were performed on male donors for whom the characteristics were available at both settings, and comparisons on unpaired data were performed on females and males donating at 700 g for whom the variables were available (see Tables 1, 2 for details). Linear regression analyses were performed on unpaired data excluding outliers. For the outcome "pre-/post-donation Δ citrate," linear regression was calculated on 61 males donating at 720 g and 54 at 700 g, and on 46 females (Tables 3, 4), and for the outcome "ACD-A re-infused to the donor," linear regression was calculated on 98 males (49 at 720 g and 49 at 700 g) and 44 females (Tables 3, 4).

Summary of the statistics and data analyses is reported in the online supplementary information. All statistical analyses were performed using STATA/SE version 16 (StataCorp LLC, College Station, TX, USA). Box plot graphs (graphs and analysis) were created using GraphPad Prism 8.4.2 (GraphPad Software Inc., CA, USA).

Sample Size

The study required at least 54 donors (providing two donations each) to achieve a power of 80% and a level of significance of 5% (two-sided), to detect a mean of the differences of 4.3 mL in reinfused ACD-A between pairs, assuming the standard deviation of the differences to be 11 mL. The difference of 5 mL in reinfused blood was that observed in our observational study [14]. Similarly, we estimated the standard deviation from the mean according to the observed standard deviation of total ACD-A used [14]. During the analyses, according to the advice received by an external reviewer, we decided to apply a multiple test adjusted significance threshold. In fact, the two co-primary endpoints measure two variables measuring a similar underlying phenomenon and contributing to the same decision-making process, i.e., the ACD-A reinfused to the donor and the increase in ACD-A in donor's blood after apheresis. Therefore, we adopted a two-sided 0.025 significance threshold according to Bonferroni's correction with two tests; considering that the two measures are not independent, the use of Bonferroni's correction is conservative. We consider the two objectives of the study, i.e., the experimental assessment of differences between 700 and 720 procedures and the observational

comparison between procedures in males and females, as two different studies, therefore, not an issue of multiple testing. Other reported p values should be interpreted as continuous variables and no prefixed threshold for rejecting the null hypothesis has been fixed.

Results

Sixty-one male donors underwent controlled plasmapheresis to collect 720 g plasma and, in the following session, 700 g plasma (online suppl. Table SI1). In parallel, 72 female donors were enrolled in the study (online suppl. Table SI1) and compared with the males in the 700 g group (Tables 1, 2). In the 700 g male group, post-donation plasma citrate values were absent for 5 donors, for which it was not possible to collect the sample. Two more values at 700 g were excluded because values fell outside the detection range of the assay (MAK057 Citrate Assay Kit, Sigma-Aldrich, USA, see Materials and Methods section and online suppl. Table SI1 for details). None of the donors recruited experienced AEs during or after plasmapheresis donation (including vasovagal reactions, nausea, tingling in the lips, malaise in general). The absence of AEs observed in the recruited donors reflects the very low percentage of AEs registered in our donor center every year (0.26% in 2020, 0.28% in 2019, 0.23% in 2018).

Male blood parameters did not change significantly between one donation and the next, except for total proteins and pre-donation citrate, which were lower when males donated at 700 g than in the previous donation at 720 g. All blood parameters differed significantly between males and females (Table 1).

The plasma unit weight setting affected most of the parameters related to the procedure. Setting the weight at 720 g instead of 700 g implied the processing of around 44 mL more total blood, corresponding to a 20 g increase in plasma collected and a higher volume of total ACD-A used (estimated by the manufacturer and measured by citrate assessment, Table 2). The ACD-A reinfused to the donor was not significantly different between the 700 g and 720 g groups. Moreover, males donating 720 g were reinfused around 12 mL more ACD-A, despite the 2 mL estimated by the collection system (Fig. 2). Measured ACD-A was also highly variable compared to the estimated values for both male groups (69.2 vs. 57.3 mL, p = 0.051, significance threshold 0.025) (Table 2; Fig. 2). Pre-/post-donation Δ citrate (Table 1) was higher in males following donation of 720 g of plasma compared with the same donors following donation of 700 g (56.4 vs. 38.6 μM, p = 0.017, significance threshold 0.025). Furthermore, the variability of pre-/post-donation Δ citrate was higher following donation of 720 g. A higher mean and greater variability in 720 g donations compared to 700 g donations resulted in a higher proportion of donors with pre-/post-donation Δ citrate higher than 100 μM (19% vs. 4%).

Sex did not significantly affect procedure-related parameters of donor groups donating at 700 g. As reported in Table 2, women were found to receive back significantly more ACD-A (67.6 vs. 57.3 mL, p = 0.004, significance threshold 0.025), with a consequently higher pre-/post-donation Δ citrate (Table 1) than males donating the same final weight of plasma (82.9 vs. 38.6 μM, p < 0.001, significance threshold 0.025). The measured ACD-A in the plasma unit was consistently lower than estimated values (Fig. 2). In women, the variability of pre-/post-donation Δ citrate was also greater than in men. Consequently, the proportion of donors with a pre-/post-donation Δ citrate over 100 μM was also higher (35% vs. 4%).

To understand which factors may affect the reinfusion of citrate-containing anticoagulant to plasma donors, we performed linear regression analysis, considering as the main outcome the difference between pre- and post-apheresis plasma citrate concentration (pre-/post-donation Δ citrate). As shown by the linear regression coefficient indicated in Table 3 for male groups, the machine weight setting and the donor HCT were significantly linked to the amount of anticoagulant reinfused to donors. Donor age also significantly impacted the increase in post-apheresis plasma citrate (and therefore of reinfused ACD-A): older donors showed a lower pre-/post-donation Δ citrate level compared to younger ones.

Finally, we investigated the factors associated with the ACD-A volume reinfused to male donors at different plasma unit weight settings (Table 3). According to linear regression analysis, the anticoagulant reinfused to donors was significantly linked to the procedure itself (namely, weight setting, total processed blood volume, total ACD-A used during the procedure), and no correlation was found with donor age and HCT.

Linear regression analysis was also applied to the female group to investigate which parameters might affect post-apheresis citrate increase and ACD-A reinfusion (Table 4). As already observed in males (Table 3), Δ citrate was found to decrease at increasing HCT and triglyceride levels, while a borderline significant association with age was shown (Fig. 2a; Table 4). In female donors, HCT was also found to be associated with ACD-A reinfusion (Fig. 2b; Table 4).

Discussion

The present study, with an experimental design involving the same donors and procedures conducted by the same personnel using the same plasmapheresis protocols, showed that a 20 g increase of the final weight of the plasma unit entails around 44 mL more of

Table 1. Characteristics of donors for which pre-/post-donation Δ citrate was available, reported as mean ± standard deviation (SD)

Characteristics	M 720		M 700		*t* test M 720 versus M 700	F 700		*t* test M 700 versus F 700
	N	mean (SD)	*N*	mean (SD)	*p* value (paired)	*N*	mean (SD)	*p* value (unpaired)
Donor								
Age	56	53.2 (7.2)	56	53.2 (7.2)		46	50.6 (8.8)	0.118
Hematocrit (HCT), %	56	42.7 (2.5)	56	43.1 (2.5)	0.129	46	40.2 (2.3)	<0.001
Estimated whole blood volume, mL	51	5,540.1 (384.1)	51	5,584.7 (520.2)	0.963	42	4,288.2 (383.5)	<0.001
Total proteins, g/dL	56	7.0 (0.3)	56	6.9 (0.4)	<0.001	45	7.1 (0.4)	<0.001
Triglycerides, mg/dL	56	119.0 (94.1)	56	109.7 (56.6)	0.388	46	90.9 (43.4)	0.038
Glucose, mg/dL	56	90.6 (12.3)	56	90.1 (14.0)	0.703	46	84.3 (7.5)	0.011
Pre-donation plasma citrate, μM	54	54.2 (24.5)	54	46.2 (19.8)	0.032	46	57.6 (25.1)	0.044
Post-donation plasma citrate, μM	54	99.2 (42.1)	54	77.2 (38.8)	<0.001	46	125.5 (49.2)	<0.001
Δ citrate in donors' plasma								
Pre-/post-donation Δ citrate, μM	54	56.4 (44.1)	54	38.6 (36.7)	0.017	46	82.9 (53.9)	<0.001

Estimated ACD-A volumes correspond to the value predicted by the plasmapheresis machine, according to the manufacturer; conversely, measured ACD-A volumes refer to the calculated amount of anticoagulant obtained from citrate quantification. The *p* value obtained from Student's *t* test on paired data for male donors and unpaired data for males versus females is reported. In bold the co-primary endpoint, for which significance threshold has been fixed at 0.025 according to Bonferroni's multiple testing adjustments.

Table 2. Parameters related to plasmapheresis procedure of donors for which the estimate of ACD-A reinfused to donors was available, reported as mean ± standard deviation (SD)

Parameters related to plasmapheresis procedure	M 720		M 700		M 720 versus M 700	F 700		M 700 versus F 700
	N	mean (SD)	*N*	mean (SD)	*p* value (paired)	*N*	mean (SD)	*p* value (unpaired)
Procedure								
Estimated ACD-A reinfused to the donor, mL	49	57.1 (15.4)	49	53.6 (8.8)	0.178	45	55.7 (3.9)	0.035
Measured ACD-A reinfused to the donor, mL	39	69.2 (31.9)	39	57.3 (15.9)	0.051	44	67.6 (16.7)	0.004*
Total volume of processed blood, mL	49	1,814.5 (76.7)	49	1,769.8 (110.0)	0.025	45	1,743.7 (46.3)	0.240
Total ACD-A used, mL	49	151.5 (6.5)	49	147.9 (9.3)	0.029	45	145.9 (4.0)	0.355
ACD-A estimated in the unit, mL	49	96.4 (3.7)	49	94.3 (3.7)	<0.001	45	90.1 (3.1)	<0.001
ACD-A measured in the unit, mL	39	79.8 (35.4)	39	91.3 (13.3)	0.084	44	78.3 (15.4)	<0.001

Paired Student's *t* test on male donor groups was performed on those for which the estimate was available for both donations, i.e., 49 donors. Of these, ACD-A measured in the plasma unit was calculated for 39 donors. Unpaired Student's *t* test between female and male groups donating at 700 g was performed on the subjects for which ACD-A reinfused to donors was available (i.e., 49 males and 45 females). In bold the co-primary endpoint, for which significance threshold has been fixed at 0.025 according to Bonferroni's multiple testing adjustments.

processed blood (Table 2). Collection efficiency is not lost, therefore, and this study does not confirm our previous hypothesis based on observational data.

With this study, we highlighted for the first time the role of the weight setting in the anticoagulant distribution between plasma unit and donor. First, we observed that

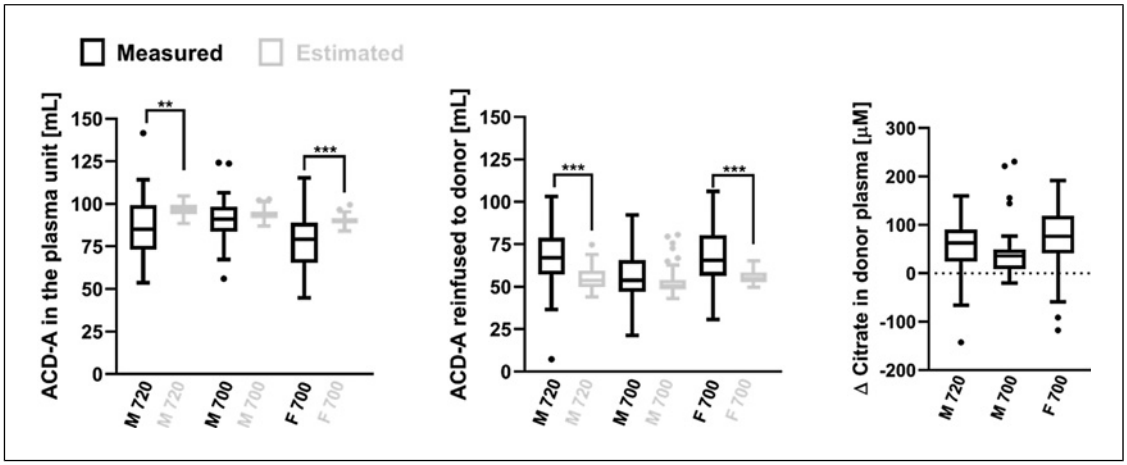

Fig. 2. Box plots of the three donor groups considered in this study (males donating 720 g of plasma – M 720; males donating 700 g of plasma – M 700; females donating at 700 g–F 700). Black box plots show the quantitative assessment of plasma citrate (measured data); gray box plots show the manufacturer's prediction (estimated data). Left panel: volume of ACD-A reinfused to donors; central panel: Δ citrate in donor's plasma (post-minus pre-apheresis plasma citrate concentration); right panel: volume of ACD-A in the final plasma unit. Asterisks indicate significant differences between measured and calculated values. ***p value < 0.001, **p value < 0.01.

the change in measured citrate concentration between pre- and post-apheresis plasma (pre-/post-donation Δ citrate) is 1.5 times higher when the final weight is set at 720 g than at 700 g (56.4 ± 44.1 μM at 720 g vs. 38.6 ± 36.7 μM at 700 g, Table 1). At the end of the procedure, 720 g donors consistently received 1.2 times more measured ACD-A than 700 g donors (69.2 ± 31.9 mL at 720 g vs. 57.3 ± 15.9 mL at 700 g, Table 2). Pre-/post-donation Δ citrate was measured on blood samples collected immediately before and after donation: since ACD-A anticoagulant is reinfused to donors throughout the process, post-apheresis citrate cannot exactly reflect the total ACD-A volume reinfused. Indeed, citrate metabolism is rapid and continuous [9], and by the time we collected post-apheresis plasma, part of the reinfused citrate might already have been metabolized. Both the estimated and measured volumes of ACD-A reinfused to donors consistently increase with the weight setting. However, the reinfused ACD-A estimated values (57.1 ± 15.4 mL at 720 g vs. 53.6 ± 8.8 at 700 g, Table 2) are underestimated compared to the measured ones (69.2 ± 31.9 mL at 720 g vs. 57.3 ± 15.9 mL at 700 g). To note, the smaller variability of the former underestimates the variance and overestimates the precision of the mean difference. An inconsistency between the estimated ACD-A and the measured ACD-A in the plasma unit was observed in a previous study conducted by our group [6]: the inconsistency in the reinfused ACD-A is just a consequence of this discrepancy.

A higher amount of measured ACD-A delivered to the donors corresponds to a smaller amount of ACD-A collected in the final plasma units. Again, the machine estimates a smaller (but significant) difference (96.4 ± 3.7 mL at 720 g vs. 94.3 ± 3.7 at 700 g) compared to the experimentally measured ACD-A in the unit (79.8 ± 35.4 at 720 g vs. 91.3 ± 13.3 mL at 700 g) (Table 2). In particular, the estimated ACD-A is significantly higher than the measured value only for the 720 g group (Fig. 2). Therefore, the plasmapheresis machine does not properly predict the ACD-A distribution, especially at higher weight settings, and this is consistent with our previously published observation on the discrepancy between estimated and measured anticoagulant in plasmapheresis units [6]. Accordingly, measured ACD-A volumes (both reinfused and collected in the plasma unit) also show greater variability than estimated values (SD in Table 2), and this is particularly evident in the 720 g group.

Since our previous observation on two donor centers was based on a mixed-gender population, to exclude (or confirm) an effect of sex on the plasmapheresis process we compared the third group of 46 female donors undergoing 700 g plasma donation with the 700 g male group (Table 1). Despite a lower pre-donation mean HCT and, consequently, less ACD-A necessary for collecting 700 g of plasma, women show a higher pre-/post-donation Δ citrate (82.9 ± 53.9 μM in women vs. 38.6 ± 36.7 μM in men at 700 g, Table 1), a higher amount of reinfused ACD-A (67.6 ± 16.7 mL in women vs. 57.3 ± 15.9 mL in men at 700 g, Table 2) and lower ACD-A in the plasma units. Furthermore, the interindividual variability in pre-/post-donation Δ citrate levels is greater in women than in men. We found that the pattern of outcomes observed in women undergoing a 700 g donation is closer to the 720 g than the 700 g male group. It is worth noting that women donating 700 g include the highest proportion of individuals with a pre-/post-donation Δ citrate over 100 μM

Table 3. Linear regression analysis of Δ citrate concentration in donor plasma and ACD-A volume reinfused to recruited males donating at 700 g and 720 g

Linear regression analysis	Pre-/post-donation Δ citrate				ACD-A reinfused to donor (measured)			
male donors	coefficient	*p* value	95% CI		coefficient	*p* value	95% CI	
Donor								
Age	−1.706	0.001	−2.723	−0.688	0.412	0.282	−0.344	1.167
Hematocrit (HCT), %	−4.494	0.005	−7.579	−1.410	1.666	0.118	−0.428	3.759
Total ACD used, mL	0.483	0.338	−0.512	1.477	1.096	0.001	0.466	1.725
Donors' blood volume, mL	−0.007	0.476	−0.026	0.012	−0.017	0.005	−0.028	−0.005
Total proteins, g/dL	7.953	0.490	−14.802	30.709	−5.378	0.478	−20.368	9.612
Triglycerides, mg/dL	−0.047	0.355	−0.148	0.054	−0.006	0.865	−0.071	0.060
Glucose, mg/dL	0.123	0.690	−0.485	0.731	−0.302	0.124	−0.688	0.084
Pre-donation plasma citrate, μM	−0.027	0.880	−0.377	0.324	0.112	0.281	−0.093	0.318
Procedure								
Plasma unit weight setting (720 g vs. 700 g)	20.354	0.008	5.336	35.372	13.112	0.015	2.640	23.584
Total volume of processed blood, mL	0.040	0.344	−0.044	0.124	0.089	0.001	0.036	0.143
ACD-A measured in the plasma unit, mL	−0.257	0.066	−0.531	0.017	−0.810	0.000	−0.940	−0.680
Measured ACD-A reinfused to the donor, mL	0.257	0.101	−0.051	0.565	–	–	–	–

Table 4. Linear regression analysis of Δ citrate concentration in donor plasma and ACD-A volume reinfused to recruited females donating at 700 g

Linear regression analysis	Pre-/post-donation Δ citrate				ACD-A reinfused to donor (measured)			
female donors	coefficient	*p* value	95% CI		coefficient	*p* value	95% CI	
Donor								
Age	−1.638	0.073	−3.436	0.160	0.349	0.184	−0.172	0.869
Hematocrit (HCT), %	−6.930	0.045	−13.682	−0.177	2.331	0.019	0.398	4.265
Total ACD-A used, mL	1.248	0.549	−2.917	5.412	1.571	0.005	0.493	2.648
Donors' blood volume, mL	−0.024	0.282	−0.068	0.020	−0.007	0.300	−0.019	0.006
Total proteins, g/dL	−7.808	0.708	−49.516	33.900	3.250	0.594	−8.934	15.434
Triglycerides, mg/dL	−0.380	0.039	−0.739	−0.020	−0.057	0.299	−0.166	0.052
Glucose, mg/dL	−0.790	0.465	−2.952	1.371	0.150	0.590	−0.406	0.706
Pre-donation plasma citrate, μM	−0.433	0.133	−1.003	0.137	−0.053	0.301	−0.156	0.049
Procedure								
Total volume of processed blood, mL	0.105	0.559	−0.255	0.465	0.122	0.013	0.027	0.217
ACD-A measured in the plasma unit, mL	−0.496	0.367	−1.594	0.601	−1.041	0.000	−1.119	−0.963
Measured ACD-A reinfused to the donor, mL	0.492	0.331	−0.518	1.503	–	–	–	–

(35% vs. 4% of males at 700 g and 19% of males at 720 g). We can thus speculate that there is also a sex effect on ACD-A redistribution between the donors and the plasma units, and that this is comparable to that observed where the machine is set to collect 20 mL more plasma. Given this result, we suggest that women donate a lower amount of plasma to ensure their safety profiles are comparable to those of men.

To explore the associations between blood donor parameters and machine settings and our main outcomes, i.e., measured ACD-A reinfusion and pre-/post-donation Δ citrate, we also built linear regression models, stratified for males and females. It is interesting to note that we found, controlling for ACD-A used and blood processed, that pre-/post-donation Δ citrate (but not reinfused ACD-A) is associated with HCT and age in the male donor population, while both correlate with HCT in females. HCT correlation was expected since it is a parameter used by the manufacturer algorithm to define the amount of ACD-A needed during the process (the higher the HCT, the higher the ACD-A used) [16]. Conversely, the association with age was unexpected: older donors showed a lower post-apheresis plasma citrate concentration compared to younger ones (Table 3), and this could be explained by an age-related change in citrate metabolism, as previously proposed [17, 18].

Unlike in males, HCT in women correlates with both reinfused ACD-A and pre-/post-donation Δ citrate,

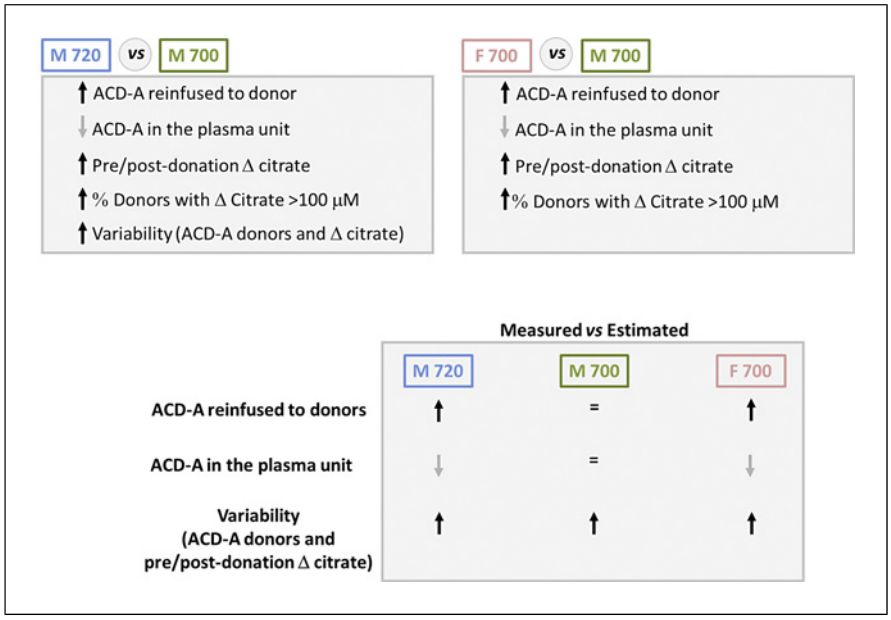

Fig. 3. Summary of the results obtained.

which is expected, given that this parameter influences the machine-guided distribution of ACD-A. The association between age and outcomes in females was weak (Table 4). Moreover, in our female group, pre-/post-donation Δ citrate (and not reinfused ACD-A) correlates significantly with triglycerides (Table 4), which also suggests a possible sex-related difference in citrate metabolism: this is in line with other published evidence [19, 20].

Figure 3 illustrates the main achievements of this study, showing clearly that both setting and sex affect the anticoagulant distribution between plasma unit and donor. Given our observations, the development of personalized plasmapheresis programs might positively impact donor safety, as proposed elsewhere [1, 7]. An updated algorithm aiming to calculate the amount of plasma to be donated might take into consideration not only the HCT but also the age and the sex of the donors. In particular, given the possible detrimental effect of long-term exposure to citrate for bone mass density [8, 12, 21–24], we suggest that special attention be paid to plasma donation in frequent female donors, who are known to undergo bone density loss more frequently than men.

We are aware of the limitations of our study: (i) post-apheresis citrate plasma concentration is affected by the timing of blood sampling [9] and by individual donor metabolism, and even slight differences in the sampling time might have contributed to making the pre-/post-donation Δ citrate values less comparable for all three donor groups. (ii) We could not randomize the order of procedures in males because it was impossible to change settings for each donation without impacting on the service organization and workload; thus, all males donated for the first time at 720 g between February and July 2020 and the second time at 700 g between June 2020 and March 2021. Consequently, the 720 g donations occurred in winter/spring while most of the 700 g donations occurred in summer/fall. Some climate or seasonal effects might have influenced the blood characteristics of the males recruited, such as total proteins and pre-apheresis plasma citrate, which were significantly higher at the time of the 720 g donation (Table 1). Females donating at 700 g, however, were recruited across the whole study period (between February 2020 and March 2021). (iii) Furthermore, since our blood donor center does not recommend setting the final weight for female donors at 720 g, we considered it unethical to perform the experimental study comparing 700 g and 720 g settings in women. (iv) Finally, our study was not powered to observe any difference in adverse events occurrence. In fact, using a maximum weight at 720 g, the occurrence of adverse events is very low: 68 events on 26,553 total plasmapheresis donations in the 3-year period from 2018 to 2020.

To conclude, we showed a sex effect on ACD-A redistribution between the plasma unit and the donor, with women receiving more ACD-A and experiencing a higher pre-/post-donation increase in blood citrate concentration than males. We also showed a higher pre-/post-donation in blood citrate concentration when the final weight of the unit is set at 720 g compared to a 700 g weight setting. Through an intragroup analysis, we also studied how donor's characteristics affect post-donation citrate metabolism in both men and women. Our results suggest that sex age and HCT should be considered for the development of tailored approaches aimed at maintaining similar safety profiles for all donors using different plasmapheresis settings.

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Statement of Ethics

The study was approved by the Ethics Committee of Reggio Emilia (Italy), on December 4, 2018 (protocol number 2018/136933), and subsequently amended on February 2, 2020 (protocol number 2020/0014263). Inclusion criteria included signed informed consent and completion of annual check-ups and plasmapheresis donations on the same day. All study participants were recruited between February 2020 and March 2021 at the Casa del Dono di Reggio Emilia, which is the main donor center of the Reggio Emilia Local Health Authority, and all provided signed informed consent forms according to the Declaration of Helsinki.

Conflict of Interest Statement

Authors declare no conflict of interest.

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Author Contributions

Lucia Merolle, Paolo Giorgi Rossi, Roberto Baricchi, and Chiara Marraccini: conceptualization. Lucia Merolle, Tommaso Fasano, Giuseppe Molinari, Agnese Razzoli, Gaia Gavioli, Davide Schiroli, Pamela Mancuso, and Chiara Marraccini: methodology. Lucia Merolle, Tommaso Fasano, Giuseppe Molinari, Agnese Razzoli, Gaia Gavioli, Davide Schiroli, and Chiara Marraccini: data dollection. Lucia Merolle, Davide Schiroli, Pamela Mancuso, Paolo Giorgi Rossi, and Chiara Marraccini: data curation. Lucia Merolle, Davide Schiroli, and Chiara Marraccini: writing – original draft preparation. Lucia Merolle, Davide Schiroli, Roberto Baricchi, Paolo Giorgi Rossi, and Chiara Marraccini: writing – review and editing. Roberto Baricchi: funding acquisition.

Data Availability Statement

All data generated or analyzed during this study are included in this article and its online supplementary material files. Further inquiries can be directed to the corresponding author.

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