Frequent source plasma donors are not at risk of iron depletion: the Ferritin Levels in Plasma Donor (FLIPD) study

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BACKGROUND: Whole blood and red blood cell (RBC) donors are at risk of iron deficiency. Since Source plasma (SP) donors have RBCs returned during apheresis, risk of iron depletion appears low. However, SP donors can donate frequently and assessment of frequent donor iron status is needed.

STUDY DESIGN AND METHODS: A total of 1254 SP donors were enrolled in four frequency groups determined by donations in the prior 12 months: no donations and 1 to 24, 25 to 69, and 70 or more donations. Ferritin was determined for each donor. Donors with ferritin levels of less than 12 ng/mL were classified as having absent iron stores (AIS).

RESULTS: Compared to new donors, ferritin for females was higher in each successive frequency group. For 70 or more donations, ferritin was 13 ng/mL higher than in new donors ($p = 0.02$). For males, 1 to 24 donations had the highest ferritin levels. Compared to new donors, highest-frequency donors had lower ferritin levels, 114 ng/mL versus 100 ng/mL ($p = 0.14$). Age for females and males increased with each successive frequency group. Age adjustment resulted in smaller ferritin differences for females and larger differences for males in the high-frequency groups; AIS for females was highest in new donors (7%) and lowest in the highest-frequency group (1%). In aggregate, AIS occurred in less than 1% of all male donors. Male new and highest-frequency donors had 1% AIS with none in the other groups.

CONCLUSION: Few SP donors have iron depletion and it is not higher in frequent donors. Frequent SP donation does not adversely impact iron stores. Thus, monitoring donor iron status or iron supplementation is not necessary.

Reduction in red blood cell (RBC) mass leading to anemia has been a recognized complication of blood donation since donor programs began after the Second World War. For many years, it was assumed that donors could be protected from an adverse impact by measuring hemoglobin (Hb) or hematocrit (Hct) before donation and setting a permissible lower limit. When it became possible to measure iron stores easily with a serum or plasma ferritin assay, Finch and colleagues in 1977[1] showed that iron stores could be reduced even without the appearance of anemia. Multiple studies confirmed this finding, demonstrating that it was especially marked in menstruating females and increased with high frequency of blood donation in males and females.[2-4] Simon and colleagues[4,5] showed that casual iron supplementation (e.g., a multivitamin) reduced this problem; regular iron supplementation with ferrous sulfate or gluconate nearly eliminated it. Because of concerns about gastrointestinal side effects of iron supplementation, Gordeuk and colleagues in the late 1980s and early 1990s[6-8] studied carbonyl iron supplementation and showed its effectiveness in female blood donors. While the emphasis has been on menstruating female donors, Garry and coworkers[9] subsequently showed that iron

ABBREVIATIONS: AIS = absent iron stores; ID = identification; PPTA = Plasma Protein Therapeutics Association; SP = source plasma.

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depletion limited the ability to donate in both male and female elderly donors.

A 2001 National Institutes of Health workshop involving major blood collection agencies concluded that further research and demonstration projects were needed before changes could be made in blood donation criteria or recommending iron supplementation to donors.[10] Ten years later publications from the REDS-II collaborative research project led to enhanced concern about the significance of this problem, particularly as it related to the impact of nonanemic iron depletion on cognition and basic life activities and its impact on young women.[11-13] Consequently, the Food and Drug Administration (FDA) with blood and plasma organizations convened another workshop in November 2011.[14] This led to an AABB Bulletin in March 2017,[15] calling for action by blood collection organizations to reduce donor iron depletion by counseling and implementing intervention strategies such as reducing donation frequency, ferritin testing, or iron supplementation of selected donors. Additional studies by the REDS-III group have shown the effectiveness of iron supplementation in recovery of Hb and iron after donation.[16]

Source plasma (SP) donors provide the starting material for the further manufacture of plasma-derived medicinal products such as albumin, immunoglobulin, and clotting factors. They donate in licensed, fixed sites which operate under standard operating procedures. In addition to medical, surgical, social, and travel history, all SP donors undergo an annual physical examination. Before each donation SP donors must meet eligibility criteria that include a screening questionnaire, vital signs, weight, Hct, and total protein tests. The minimum age for SP donation is 18.

Most of the donations are from frequent donors with continued recruitment of new donors due to growing demand. SP released for manufacturing, however, comes exclusively from qualified donors who must pass separate medical screenings and testing for human immunodeficiency virus, hepatitis B virus, and hepatitis C virus on two different occasions. Generally, the pool of SP donors does not overlap the pool of whole blood donors or component apheresis donors from community blood centers. There were more than 38 million SP collections in 601 US donor centers in 2016. SP donors must meet the same Hb and Hct requirements as whole blood donors to give plasma. During plasmapheresis, SP donors receive their RBCs back; thus, iron stores should not be affected. In addition, all testing performed uses plasma samples obtained from the collected plasma unit, except for serum protein electrophoresis and syphilis screening tests which are tested from a whole blood sample collected initially and every 4 months. SP donors are eligible to donate up to two times in a 7-day period. For very frequent donors, the cumulative effect of a small RBC loss at each donation could result in a total RBC loss equivalent to many blood donors, who can donate every 56 days. With the current automated SP collection devices, each donation could lose an estimated 10 to 11 mL of RBCs without postdonation saline reinfusion. Saline reinfusion rinses the collection device and results in residual RBCs being reinfused, minimizing the loss to 5 mL or less per donation. Most US collection procedures include saline replacement. Thus, for example, a donor donating plasma 50 times per year could lose 250 mL of RBCs annually. Thus, at the 2011 workshop, the plasma industry was encouraged to assess iron status of frequent donors. Therefore, the Plasma Protein Therapeutics Association (PPTA), the association representing almost all US SP collectors, organized a study of donors with different donation frequencies to assess iron status and whether there was evidence that more frequent donation reduced iron stores.

MATERIALS AND METHODS

Three US SP donation centers participated in the study: BioLife Plasma Services, L.P., St George, UT; CSL Plasma, Lansing, MI; and Talecris Plasma Resources (a Grifols, Inc. subsidiary), Flint, MI. Two centers used Haemonetics (Model PCS and PCS2) and one center Fresenius Kabi (Autopheresis-C) automated apheresis devices for plasma collection. Each center collected plasma samples from approximately the same number of preselected donors in each donation frequency category for analysis of ferritin.

Study participants

The targeted minimum number of donors for each donation frequency was 150 females and 125 males. A larger number of females is necessary because females in the general population have lower ferritin levels.[17] Centers oversampled for each group to compensate for possible inadequate samples and misclassification of donation frequency.

Plasma samples from 1254 donors were analyzed for ferritin level ($\geq$400 donors/center). Donors were assigned to one of four groups, according to their frequency of plasma donation in the 12 months before enrollment. The frequency groups were chosen to provide significant differentiation between the donor groups in donation frequency and to enable recruitment of donors to take place over a reasonable time period. Donation frequency assignment was made using the center's donor management system when the donor made an appointment to give plasma. After enrollment, final frequency assignments occurred after checking the donor's history. There was no other recruitment of donors for the study.

The study protocol was approved by an independent institutional review board, Schulman Associates (Cincinnati, OH). After donors gave written informed consent for participation they were enrolled in the study and provided

a blood sample for the ferritin assay. Donors received no compensation for study participation.

Subject enrollment

A random sample of qualifying donors was selected and allocated to the appropriate frequency group until the required number of donors was enrolled. Once this occurred, blood samples and information were collected only for individuals with donation frequencies matching groups not filled. Enrollment logs were kept by the center containing the plasma center donor identification (ID) and the study ID sequentially assigned within each frequency group. Upon completion of enrollment, only the study ID was transmitted to PPTA, the coordinating institution. This served to deidentify subjects and protect confidentiality. The study's four donation frequency groups included the following donors.

New donors, consisting of first-time and reactivated donors who had not donated in the prior 12 months, were identified either when accepted as first-time donors (in accordance with the center's standard operating procedures) or through the donor management system as not donating plasma in the prior 12 months. The selection of "new donors" was based on the order in which they were accepted as a first-time or reactivated donor and a daily enrollment quota.

Low-frequency donors (1-24 donations in prior 12 months), high-frequency donors (25-69 donations), and very-high-frequency donors ($\geq$70 donations in prior 12 months), consisting of current donors making at least their second donation, were identified based on donations in the prior 12 months through the center's donor management database. Enrollment continued until the desired number of donors was achieved in each group. After enrollment, centers checked the prior 12-month donation frequencies and, if needed, reassigned donors to the proper frequency group.

Routinely collected donation information was extracted for each donor from the center's donor management system. This included donor demographics (sex, weight, and age), Hct, and donation history. The Hct was a directly measured spun Hct using a capillary fingerstick sample.

Donor enrollment procedures (Fig. 1)

Donors were eligible to participate after successful completion of the predonation health history and physical examination. There were no selection criteria for age; 18 is the minimum age for plasma donation. Donors were eligible for the study only once.

Exclusion criteria were: donation history indicating a recorded RBC loss in the past 8 weeks or a deferral due to more than 200 mL of RBC loss in the prior 12 months (generally due to an incident in the collection procedure that prevented the reinfusion of RBCs) at the participating center or at any center under the same corporate ownership, had donated whole blood within the past year,

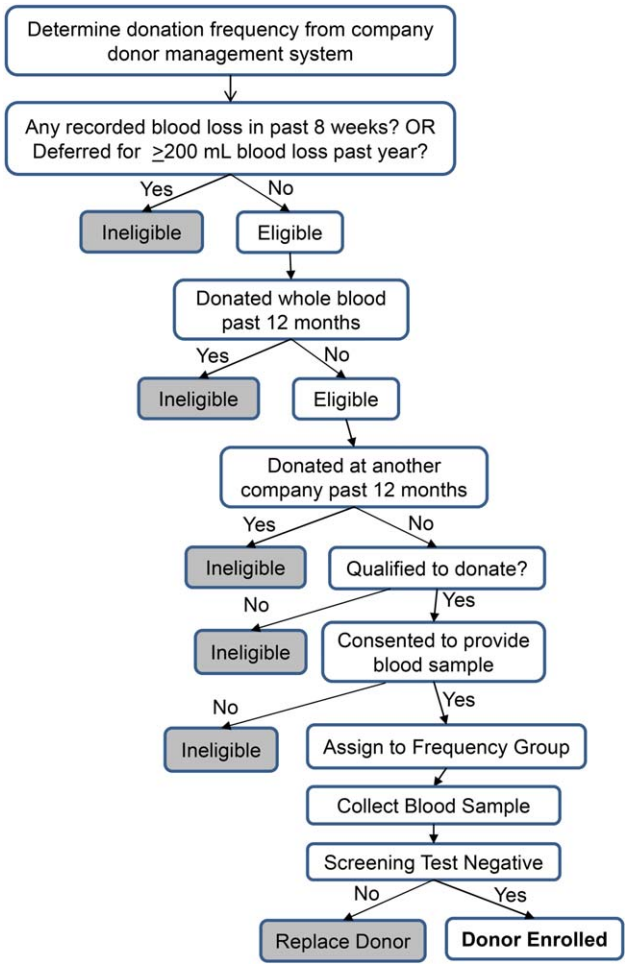

Fig. 1. Donor study enrollment. [Color figure can be viewed at wileyonlinelibrary.com]

donated plasma within the past year at a center under different ownership, tested repeat reactive on any serologic test or reactive on any nucleic acid test (NAT), failed to meet any of the requirements to be a SP donor, or had enrolled in the study already. The exclusions related to RBC loss and whole blood donation were in place to assure that the study measured the consequences of routine SP donation.

Since the results of the infectious disease serology and NATs are not immediately available, if an enrolled donor was subsequently found to be positive on any infectious disease screening test, the donor was excluded from the analysis since they did not qualify as a donor and the impact of a viral infection on ferritin is also unknown. Any donor with a positive screening test result was removed from the study by the center and a replacement enrolled if the quota had not been met.

Sample collection procedures

A separate venipuncture was not required for the ferritin assay. At the beginning of the donor's routine plasma

TABLE 1. Donor frequency groups

Donor frequency group	Number of donations prior 12 months	Number enrolled in study	Female*	Male*	Age (years) Female	Age (years) Male
No prior donations ("new donors")	0	309	164 (53)	145 (47)	28.7	28.4
Low-frequency donors ("low")	1-24	306	168 (55)	138 (45)	33.7	34.6
High-frequency donors ("high")	25-69	342	181 (53)	161 (47)	37.5	38.3
Very-high-frequency donors ("very high")	≥70	297	156 (53)	141 (47)	43.0	42.6
All	Any	1254	669 (53)	585 (47)		

* Data are reported as number (%).

donation, a whole blood sample in a 4.5-mL heparin plasma separator vacutainer was collected. The vacutainer was labeled with a study ID and inverted gently eight times to ensure mixing of anticoagulant with blood to prevent clotting.

The plasma was separated from the cells by centrifugation as soon as possible, but within 2 hours of collection. The sample was examined; if grossly hemolyzed the donor was excluded and a replacement donor enrolled. The plasma fraction was transferred by decanting approximately 2 mL, but no less than 0.5 mL, of plasma to a standard storage and transport tube (ARUP Laboratories). If a minimum of 0.5 mL of plasma could not be obtained the donor was excluded from the study and a replacement enrolled. Immediately after plasma decantation the tube was frozen at not more than –20°C. Samples were shipped frozen by overnight delivery to the analytical laboratory.

Ferritin assay

A commercially available quantitative chemiluminescent immunoassay ferritin assay (ADVIA Centaur, Siemens Healthcare Diagnostics) was performed (ARUP Laboratories, Salt Lake City, UT).

Statistical analysis

Data processing and statistical analyses were performed by Statistics Collaborative (Washington, DC). Analyses were conducted separately by sex since normal ferritin concentrations are considerably lower in females than in males. Assumptions included normal ferritin concentration ranges of 12 to 150 ng/mL with a mean ± SD of 25 ± 25 ng/mL for females and 12 to 300 ng/mL with mean ± SD of 125 ± 50 ng/mL for males.[17] With one-sided Type I error rates of 0.0125 (or two-sided 0.025), sample sizes of 150 females and 125 males for each of the four donation groups provided approximately 90% power to rule out decreases of 11 ng/mL in females and 23 ng/mL in males when comparing the low- and high-frequency donation groups to the new donor group.

The distributions of ferritin by donation frequency are skewed due to some high outliers. Medians, which are similar to geometric means, and means show similar conclusions in ferritin level trends across the donation groups. Differences in means between each donation group and the no-prior-donation group were compared using a two-sample t test. Within sex, the influence of outliers on summary statistics and confidence intervals (CIs) was explored by setting observations above the 95th or 99th percentiles to that value. Ninety-five percent CIs were used to assess whether ferritin levels for frequent donors were no worse than, or within the margin of noninferiority, when compared to new donors.

To adjust for the age difference in the donation groups, analyses used general linear regression with ferritin level as the outcome and donation and age group (cutoff at 50 years) as categorical covariates. For women, this served as a proxy cutoff for pre- and postmenopausal status. Separate models were constructed for males and females, with resulting ferritin level model estimates and their 95% CIs adjusted for age and donation group. All inferences used two-sided Type I error rates of 0.05 (or one-sided 0.025). Analyses were not adjusted for multiple comparisons.

Robust regression, which reduces the potential influence of outliers, was also performed as a sensitivity analysis. While the point estimates of ferritin by donation group differed somewhat across these analyses, the inferences for noninferiority remained the same as the primary predefined analysis. Regression using the log transformation of ferritin was also performed. All analyses were performed with computer software (SAS 9.4, 2013, SAS Institute, Inc.).

RESULTS

There were 1254 donors enrolled in the study. A total of 1328 donors were asked to participate, 51 of whom refused, for a participation rate of 96%. Subsequent to enrollment five donors decided to disenroll. In addition,13 donors were excluded after enrollment. Of these, four did not meet company requirements for donation, four had no sample collected, two were reactive on infectious disease testing, and three were excluded for other reasons.

Table 1 shows the number of donors in each frequency group. Each group had approximately 300 donors

TABLE 2. Mean ferritin level and unadjusted and age-adjusted differences between frequency groups and new donors, by sex and donation frequency*

			Unadjusted			Adjusted†		
Donor group	Number	Ferritin level (ng/mL)	Low/high, new differences	Within noninferiority margin‡	p value (two-sided)	Low/high, new differences	Within noninferiority margin‡	p value (two-sided)
Females								
New donors	164	51.1 ± 41.2	0	—	—	0	—	—
Low	168	53.3 ± 49.8	2.2 (–7.7 to 12.1)	Yes	0.66	0.6 (–10.0 to 11.2)	Yes	0.91
High	181	57.5 ± 52.9	6.4 (–3.7 to 16.5)	Yes	0.22	3.2 (–7.2 to 13.7)	Yes	0.54
Very high	156	64.0 ± 57.3	13.0 (2.0 to 23.9)	Yes	0.02	3.5 (–7.7 to 14.6)	Yes	0.54
Males								
New donors	145	114 ± 73.4	0	—	—	0	—	—
Low	138	120 ± 75.8	5.7 (–11.8 to 23)	Yes	0.52	3.2 (–15.0 to 21.3)	Yes	0.73
High	161	111 ± 77.9	–3.7 (–20.8 to 13)	Yes	0.67	–8.1 (–25.8 to –9.5)	No	0.37
Very high	141	100 ± 85.5	–13.9 (–32.4 to 4.6)	No	0.14	–21.3 (–39.9 to –2.7)	No	0.03

* Data are reported as mean ± SD or number (95% CI).
† Estimates, CIs, and p values are based on the coefficients from a regression model with donation frequency group and age group (<50 and ≥50 years) as categorical covariates.
‡ No worse than new donors (within noninferiority margin).[28]
A donor group is considered within the margin compared to the no prior donation (new donor) group if the lower bound of the CI lies above –11 ng/mL for females and –23 ng/mL for males.

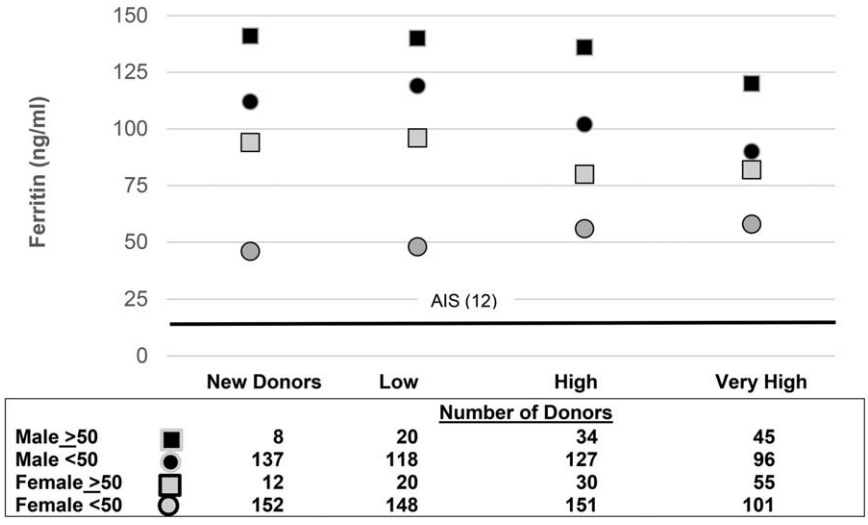

Fig. 2. Ferritin level, by sex and age.

who completed the study (297-342) with a female-to-male ratio of 53:47 except for the low-frequency group, which had a female-to-male ratio of 55:45.

Mean age steadily increased from 28 years in the new donor group to 43 in the very-high-frequency donor group for both females and males (Table 1). This reflects the increasing percentage of older donors with each donation frequency group. Mean Hct varied little across frequency groups and was approximately 42% for females and 46% for males in each of the four groups. The largest difference between the highest and the lowest Hct groups was 0.2% for females and 0.3% for males. Mean numbers of donations for females and males were similar in each donation frequency group: 13.2 ± 6.3 (females) and 14.4 ± 5.9 (males) in the low-frequency group, 45.6 ± 13.1 (females) and 48.1 ± 14.4 (males) in the high-frequency group, and 84.2 ± 9.1 (females) and 87.7 ± 9.5 (males) in the very-high-frequency group. The correlation between Hct at

TABLE 3. Observed ferritin levels, by sex, donation frequency, and age group

Sex and donation frequency	Overall				Age < 50 years				Age ≥ 50 years			
	Number	Ferritin level (ng/mL)*	Median	Geometric mean	Number	Ferritin level (ng/mL)*	Median	Geometric mean	Number	Ferritin level (ng/mL)*	Median	Geometric mean
Females												
New donor	164	51.1 ± 41.2	39	37.9	152	47.8 ± 37.3	37	35.9	12	92.8 ± 63.7	71	75.3
Low	168	53.3 ± 49.8	40	40.0	148	47.6 ± 36.3	39	37.8	20	94.9 ± 97.4	64	60.6
High	181	57.5 ± 52.9	44	43.2	151	52.9 ± 47.2	39	40.8	30	80.5 ± 72.3	52	58.0
Very high	156	64.0 ± 57.3	48	48.5	101	54.0 ± 45.0	43	42.1	55	82.4 ± 71.8	67	62.6
Males												
New donor	145	114 ± 73.4	100	92.6	137	113 ± 73.8	98	91.1	8	143 ± 64.7	162	121.5
Low	138	120 ± 75.8	100	100.2	118	117 ± 70.1	97.5	99.1	20	139 ± 104	115	106.5
High	161	111 ± 77.9	86	90.4	127	105 ± 72.4	85	86.7	34	133 ± 93.8	103	105.6
Very high	141	100 ± 85.5	79	78.1	96	90.9 ± 67.5	72	73.7	45	121 ± 113	85	88.4

* Data are reported as mean ± SD.

TABLE 4. Donors with AIS (ferritin < 12 ng/mL), by donation frequency and sex

Sex	New donors	Low AIS/N	High AIS/N	Very high AIS/N	Total AIS/N
Females	12/164 (7%)	9/168 (5%)	5/181 (3%)	2/156 (1%)	28/669 (4%)
Males	1/145 (1%)	0/138 (0%)	0/161 (0%)	2/141 (1%)	3/585 (<1%)

donation and number of donations in the prior 12 months was very low ($r < 0.03$ for both sexes), as was the correlation with ferritin level ($r = 0.11$ for females, $r = –0.02$ for males), illustrating that within the FDA-acceptable range Hct is not associated with donation frequency and is a poor indicator for ferritin levels.

Among females, mean ferritin (Table 2) was actually higher with increased donation frequency. The difference between the new donor and very-high-frequency group was significant ($p = 0.02$). Among males, ferritin was highest in the low frequency group, and slightly lower than the new donors in the high-frequency group. The difference in ferritin concentration was largest in the very-high-frequency group for males. The ferritin differences, however, were not significant ($p = 0.67$ and $p = 0.14$, respectively).

Figure 2 shows the relationship of ferritin with sex and age. In each age group ferritin was greater among males than females for all frequency groups. Males 50 years and older had higher ferritin levels at each donation frequency than males less than 50 years. The same age pattern was seen for females. The ferritin levels in the very-high-frequency donors for females 50 years and older (82.4 ± 71.8 years) were almost equal that of males age less than 50 (90.9 ± 67.5 years). Ferritin levels by sex, donation frequency, and age group are shown in Table 3. A higher proportion of donors 50 years and older were present in the more frequent donation groups. For females, 7% of those with no prior donations were at least 50 years of age, while 35% of the highest-frequency donors were 50 years and older. Similarly, for males, 6% of those with no prior donations were 50 years and older. For the most frequent male donors, 32% were at least 50 years of age.

TABLE 5. Median ferritin and percent of donors with AIS, for SP donors (FLIPD) and whole blood donors (RISE[12]), by donation frequency and sex

Donor group	Median ferritin (ng/mL)		AIS (%)	
	New donors	High frequency*	New donors	High frequency*
Females				
SP	39	45	7.0	2.1
Whole blood	37	19	6.4	27.1
Males				
SP	100	84	1.0	0.7
Whole blood	108	25	0.0	16.4

* High frequency: SP: ≥ 25 donations in 12 months; whole blood (RISE[12]): females ≥ 2 donations in 12 months, males ≥ 3 donations in 12 months.

The history of donating plasma increased with each successive donation frequency group. Female plasma donors making 1 to 24, 25 to 69, and 70 or more donations had donated on average for 26.4, 39.0, and 42.0 months, respectively, and males for 29.2, 39.3, and 48.0 months, illustrating their commitment to regular plasma donation.

With the association of ferritin with age, and the observed increase in age with frequent donors, a model adjusts for the effect of age on the differences between ferritin in the frequency groups and the new donor group (Table 2). After age adjustment, the differences decreased for females for each comparison, but were not significant.

For males, adjustments magnified the differences for the two high-frequency groups due to the age mix and higher percentages of older donors in the high-frequency groups. Differences were significant for the very-high-frequency male donors only ($p = 0.03$). Results from models using log-transformed ferritin had directions of effect and inferences that were consistent with results from nontransformed data. This applied also to models adjusting for age.

Absent iron stores (AIS) were defined as a ferritin level of less than 12 ng/mL.[11] Twenty-eight female donors met the criterion with the highest numbers in the new donor (12 or 7%) and the low donation (9 or 5%) categories (Table 4). Only five female donors in the high-frequency category (3%) and two in the very-high-frequency category (1%) met the criterion for AIS. AIS were only seen in three male donors. One was a new donor and two were in the very-high-frequency group. Overall less than 1% of male donors had AIS.

Table 5 compares both median ferritin level and AIS for males and females in new donors and in both high-frequency donor groups combined in this study with high-frequency donors in the RISE study of frequent whole blood donors.[11] This shows the substantial contrast in ferritin levels and AIS between frequent blood donors and plasma donors. Both female and male frequent blood donors had 1) substantially lower ferritin levels than frequent plasma donors and 2) were much more likely to have AIS than frequent SP donors.

DISCUSSION

The 1254 SP donors enrolled in this study were from three donor centers operated by different companies and were almost evenly divided into four groups defined by donations made in the prior 12 months: new donors with no donations, low-frequency donors with 1 to 24 donations, high-frequency donors who donated 25 to 69 times, and very-high-frequency donors with 70 or more donations. In the United States, donors are allowed to donate by FDA regulations two times in 7 days with a minimum of 1 day between donations. However, this maximum frequency is rare. Automated apheresis is used to collect plasma, the amount of which is in accordance with a weight-varying nomogram established by the FDA.[18] Our analyses have not factored in volume of plasma donated, but more frequent donors typically make larger-volume donations. Therefore, ferritin differences in the very-high-frequency males could possibly in part result from higher volume donations.

All plasma donors received saline at the end of the procedure that rinses RBCs in the tubing back into the circulation. This process attenuates RBC loss, which is already low since RBCs are returned to the donor. SP donors are drawn only at fixed collection sites. The mean ferritin values and proportion of new female donors with AIS for our SP study was comparable to, or even more favorable than, reported in US population-based National Health and Nutrition Examination Study findings.[17,19,20] The minimum age for plasma donation is 18 years; thus, the number of high school students would be much lower than in whole blood programs, which generally accept 16- and 17-year-old donors at high school blood drives. Thus, our finding that the lower AIS observed among new donors, compared to many reports from blood centers[21-23] may be partially explained by the exclusion of the youngest donors, especially young females. While our protocol called for excluding donors with RBC loss and recent whole blood donors to allow us to assess the impact of SP donation in the United States by itself, uninfluenced by additional RBC loss, no donors asked to participate in this investigation met the exclusion criteria. We do not therefore believe that the lower rate of AIS was influenced by donor selection. Individuals who had experienced blood loss could have lower ferritins, but this is a rare occurrence.

Donors are screened for Hct before donating. Most deferrals for low Hct are first-time donors and likely to be female. They might have lower ferritin levels, but if they were included in our new donor group the mean ferritin levels would have in fact been lower. With the low deferral rates in the frequent donors the ferritin findings would probably not be noticeably impacted. The comparisons with the three donor frequency groups would have then resulted in smaller ferritin differences.

Younger donors were more prevalent in the new donor group. The mean age increased with increasing donation frequency. Mean Hct for females and males was the same in each donation group (approx. 42% females, 46% males). In addition, the correlation between donation frequency and Hct was very low ($r < 0.03$ for both sexes). This suggests that frequency of donation has minimal impact on the Hct. Taken together, the Hct and ferritin data support the fact that frequent SP donation does not result in reduced iron stores and thus anemia. Donors with deferral for low Hct also may self-select to stop donating. This could contribute to the observed female increase in ferritin with donation frequency, since there is no known physiologic mechanism associated with plasmapheresis donation to increase iron stores. Center personnel advice to donors to adhere to a good, well-balanced diet could also help maintain iron stores. Use of iron supplements could not be assessed although it is not a routine recommendation for plasma donors.

There was an impact of age on the ferritin levels. Males 50 years and older had higher ferritin levels than males less than 50 years of age. Ferritin levels decreased slightly in the higher-donation-frequency groups, but mean values were well within the normal range, and the differences did not approach significance. Conversely,

females 50 years and older had higher ferritin levels than younger females. This is consistent with the recovery of iron stores after menopause, contributing to the observed higher ferritin levels seen in frequent donors since the average age is older than that of new donors.

Controlling for the donor age did result in a significant difference between new and the very frequent donors for males, but not females. This illustrates the importance of age in iron metabolism. Even after age adjustment, however, the mean ferritin levels for the most frequent donation group are well within normal ranges, supporting our findings that the prevalence of AIS is rare among those donating the most frequently.

The analysis of ferritin usually uses geometric means and log transforms because of the skewed nature of the data. The subsequent interpretation for changes between groups involves ratios and percent changes, rather than differences. Our focus was a noninferiority design. We felt that the interpretation of a noninferiority margin using arithmetic differences was more intuitive than one using ratios. Although the interpretation of regression results between the two methods cannot be directly compared for this reason, the inferences for changes between each group and new donors were consistent. With either method, the direction of effect was the same, and inferences based on significance were also unchanged.

A concern raised is that reduction in plasma iron by the apheresis process could contribute to iron deficiency. Our ferritin data clearly indicate no such impact, substantiating that circulating plasma iron represents a small portion of total body iron. Plasma contains on average 4 mg of iron in comparison to 250 mg in hepatocytes, 500 mg in macrophages, 2500 mg in RBCs, and 150 mg in bone marrow. Tissue's store of iron is in iron containing enzymes (≈150 mg) and myoglobin (approx. 300 mg). Redistribution of iron to replenish circulatory plasma iron bound to transferrin can be easily achieved.[24]

The concern for iron deficiency in blood donors has resulted in various strategies to protect donors' health in the United States and elsewhere. These include providing information to donors about iron deficiency and steps to prevent it, increasing the interdonation interval, testing ferritin before donation, and providing iron supplementation. The last measure has been shown in controlled clinical trials to protect frequent blood donors from iron depletion with the result that both anemia and nonanemic consequences of iron depletion are prevented.[25] Iron depletion has also been reported in apheresis donors, particularly platelet apheresis donors.[26,27] In the face of increasing attention to this problem by community blood centers and regulatory authorities, we believe our data are important and provide evidence that no further action by SP donor centers is needed to ensure adequate iron stores.

From our findings, frequent SP donation in the United States does not adversely impact iron stores or Hct in donors. Fluid replacement minimizes iron and RBC loss from the plasmapheresis process by returning residual RBC to the donor. Thus, in contrast to whole blood donation and reports about frequent plateletpheresis, plasmapheresis in the United States is not associated with increased iron depletion nor anemia. It also suggests that the nutritional status of SP donors is adequate with regard to iron.

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CONFLICT OF INTEREST

GBS is an employee of the Plasma Protein Therapeutics Association; RB is an employee of the BioLife Plasma Services LP/Shire; MRB is an employee of Grifols Plasma Operations; ZFY provided statistical analysis and consulting for this study for PPTA through her employer, Statistics Collaborative, Inc.; and TS is an employee of CSL Plasma.

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