CHAPTER 5

Adverse reactions and iron deficiency after blood donation

Anne F. Eder[1] & Joseph E. Kiss[2]

[1] National Medical Affairs; Biomedical Services, National Headquarters, American Red Cross and Adjunct Associate Professor, Georgetown University Medical Center, Washington, DC, USA

[2] Hemapheresis and Blood Services, The Institute for Transfusion Medicine; and Associate Professor of Medicine, Division of Hematology-Oncology, Department of Medicine, The University of Pittsburgh, Pittsburgh, PA, USA

A safe and adequate blood supply depends on healthy, altruistic volunteers who are willing to donate blood without expecting personal gain despite the potential risk of discomfort or adverse reaction. Blood donation has an impressive safety record, and most donors have a good experience or only mild symptoms after donation. But with over 9 million blood donations each year in the United States,[1] even a low rate of reactions may negatively affect the health and well-being of many people and influence their willingness to donate again. Adverse reactions can occur immediately or soon after a blood collection procedure or become apparent only after multiple, regular blood donations over an extended period of time. Immediate or acute reactions after blood donation typically are minor symptoms such as dizziness and fainting or phlebotomy-related bruises, but also the less common and more serious injuries; long-term or chronic complications include iron depletion and its sequelae. Blood collection agencies must inform prospective donors about the possible risks at each encounter and monitor adverse reactions during and after the collection procedure as part of ongoing continuous improvement efforts to reduce donation-related complications. Although blood centers cannot completely eliminate all risks associated with blood donation, the systematic analysis of adverse reactions has led to changes in collection procedures and policies that have significantly improved safety for the most susceptible groups of blood donors.

Adverse reactions after blood donation

Most acute reactions (>90%) that occur immediately or within a few hours after donating blood are mild or minor symptoms such as dizziness, lightheadedness, or phlebotomy-related bruises and hematomas that resolve promptly but are still unpleasant for the donor. More serious complications are uncommon, but typically result from a loss of consciousness, from injuries after blood donation, or from a needle-related nerve injury.[2] Young donors are much more likely to experience immediate adverse reactions after blood donation than older donors, with loss of consciousness affecting about 4 in 1000 donations and injury resulting in 0.6 in 1000 donations among donors age 16 to 17 years.[3] Not surprisingly, blood donors who have an adverse reaction are less likely to return to donate blood again than those who have an uneventful donation.[4–6] Even minor reactions and transient symptoms discourage return donation by 36%, with more severe reactions further decreasing the likelihood by 66%.[4] The potential loss in annual donations resulting from donor attrition after adverse reactions has been estimated as 1.6% per year.[5] Donor retention has far-reaching implications for the blood supply, for not only availability but also safety because repeat donors account for about 70% of the US blood supply and are less likely than first-time donors to have positive infectious disease markers, such as hepatitis and HIV.

Many studies of reactions after allogeneic blood donation published since the 1940s have reported various incidence rates and associated risk factors.[7] The observed reaction rates in different blood centers span a wide range (<1% to >20%). Many factors contribute to this variability, including different reaction definitions, subjective assessments of reaction severity, disparate donor demographics, and various methods of data collection and analysis. Reactions may be captured at the site by collection staff or identified only after the donor calls the center back to report a reaction. Higher reaction rates are generally observed when donors complete surveys asking them about mild subjective symptoms or are directly interviewed days to weeks after a donation.[8,9] Many studies of donor reactions are limited by methodological problems, including retrospective design, poorly controlled comparisons, or the use of univariate methods to detect associations of various factors with donor reactions. Consequently, any conclusions about the possible association of donation-related reactions with various risk factors should be evaluated in conjunction with an assessment of the study design and analytical methods.

In recent years, blood centers have focused on the practical application of donor hemovigilance, which is an effort to monitor, track, and trend reactions after blood donation, in order to design and implement preventive measures. An example of such a program in the American Red Cross is shown in Table 5.1, which captures reactions managed by collection staff at the blood drives and calls back to the blood center about delayed reactions after whole blood and automated (apheresis) blood donation. AABB has also launched a voluntary national program for donor hemovigilance in the United States, which encourages centers to use common reaction definitions and may facilitate analysis of complicated data

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Table 5.1 Adverse reactions after allogeneic whole blood and apheresis donations in the American Red Cross, 2006–2007

Reaction Category and Description		Whole Blood Donations (12.0 Million)				Automated Procedures (1.42 Million)			
		All Reactions		Outside Medical Care		All Reactions		Outside Medical Care	
		n	Rate	*n*	Rate	*n*	Rate	*n*	Rate
Systemic (syncopal)	Presyncope (prefaint)	324,129	269	69	0.06	14,919	104	4	0.03
	LOC (<1 min)	11,081	9.20	107	0.09	521	3.65	4	0.03
	Major (includes callbacks)								
	LOC (≥1 min)	2050	1.70	251	0.21	206	1.44	14	0.10
	Prolonged recovery	4228	3.51	829	0.69	424	2.97	48	0.34
	LOC with Injury	2181	1.81	680	0.57	98	0.69	18	0.13
Phlebotomy	Small hematoma	125,082	104	87	0.07	49,304	345	13	0.09
	Major (includes callbacks)								
	Large Hematoma	4932	4.09	556	0.46	2850	19.9	103	0.72
	Suspected nerve injury	3858	3.20	513	0.43	572	4.00	47	0.33
	Suspected arterial puncture	1644	1.37	112	0.09	82	0.57	1	0.01
Citrate reactions	Citrate (minor symptoms)	—	—	—	—	16,556	116	3	0.02
	Citrate (major, includes callbacks)	—	—	—	—	406	2.84	21	0.15
Allergic reactions	Local (minor) allergic reactions	123	0.10	19	0.02	49	0.34	8	0.06
	Systemic (major) allergic reactions (includes callbacks)	17	0.01	11	0.01	42	0.29	8	0.06
	TOTAL	479,325	398	3234	2.69	86,029	602	292	2.04

Rate per 10,000 donations. LOC, loss of consciousness; small hematoma, 2 × 2 in. or less; large hematoma, more than 2 × 2 in. Prolonged recovery (>30 minutes) after presyncope
Automated collections include plateletpheresis, plateletpheresis with concurrent plasma or other co-component, 2-unit red cell collections, and plasmapheresis procedures
Minor reactions (e.g., presyncope, small hematoma) are documented at the collection site; Major reactions are documented at the collection site or reported after donation, require follow-up with the donor, and receive a follow-up call, and are reviewed by a blood center physician.

sets. Although the absolute incidence of donor complications varies dramatically among centers for all the reasons mentioned previously, the practical value of hemovigilance activities lies not in the casual comparison or "benchmarking" of reported rates across blood centers, but in the careful analysis of rates within a blood system as part of continuous process improvement efforts to monitor and ultimately reduce the risk of reactions over time.

Acute reactions after blood donation: immediate symptoms and delayed complications

Most systemic reactions (95%) to blood donation are acute symptoms such as pallor, lightheadedness, dizziness, diaphoresis, and nausea, occurring in about 2–15% of whole blood donations.[2] The term *vasovagal* is often used to describe these reactions, to refer to physiologic changes that may be associated with syncope (i.e., increased vagal tone and bradycardia). The more general term *presyncope* captures the spectrum of reactions that result from various mechanisms involving not only peripheral baroreceptor activity, but also susceptibility to acute blood loss and orthostatic changes, as well as anxiety and psychological stress. Ultimately, syncope results from an insufficient supply of oxygen to the brain and a transient loss of consciousness. About one in every 20 presyncopal reactions (Table 5.1) progresses to loss of consciousness immediately or soon after the blood donation, and may be associated with seizure-like movements or loss of bowel and bladder function.

Presyncopal symptoms typically have rapid onset and short duration, and they resolve spontaneously. Although most of these reactions are self-limiting and transient, they are still distressing for many donors and some require prolonged recovery periods of more than 30 minutes.[2] Syncope-related falls may be associated with serious injuries. Most (90%) fainting events occur with the phlebotomy or soon afterward at the blood drive, but delayed hypotensive reactions after the donor left the collection site comprised about 10% of the episodes in one series.[10,11] Young donors (16 to 17 years old) accounted for almost half of all injuries at collection sites among whole blood donors, and some of the injuries (e.g., concussions, lacerations, and dental injuries) required urgent medical care.[3]

Risk factors associated with reactions after blood donation

Many studies have evaluated donor characteristics and other variables that influence the risk of immediate and delayed reactions after allogeneic blood donation, and the strength of the conclusions depends on the study design and statistical analysis to control for confounding variables. An overview of risk factors and the strength of the available evidence that supports a possible association with syncopal reactions after allogeneic blood donation is summarized in Table 5.2.[7] The strongest, independent risk factors for both immediate and delayed reactions consistently identified in several well-controlled studies of whole blood donation are young age (<23 years old), first-time donation status, total blood volume (<3.5 L) and estimated blood loss (>15%), and, in most studies, female sex.[12–14] Young age had the strongest association with complications even after controlling for first-time donation status in one study that showed 16 and 17 year olds were threefold more likely to experience an adverse reaction than older donors.[3] Although regulations in some countries restrict the upper age limit of blood donation, many studies have confirmed the lower observed rates of reactions among elderly donors compared to younger donors.[15,16] A multivariate analysis predicted that young donors (<23 years) with low blood volume contributed about 3% of all donations but disproportionately accounted for about 10% to 15% of presyncopal reactions and syncope-related complications after whole blood

Table 5.2 Variables associated with syncopal reactions after blood donation

Independent variables strongly associated with increased risk (strong evidence based on multivariate analysis in multiple studies):
- First-time vs. repeat donation status
- Young (<23 years) vs. older age
- Low body weight/total blood volume (<3.5 L) with standard WB donation (~525 mL)
- Female vs. male sex
- Caucasian vs. African ethnicity

Variables associated with increased risk (weak or no association in some studies, inconsistent or low-quality evidence, and poorly controlled or univariate analysis)
- Admitted anxiety
- Collection volume
- Greater than 4 hours since last meal
- Temperature/season
- Wait time
- Duration of phlebotomy
- History of fainting not related to blood donation
- Mobile blood drive
- History of prior reactions after blood donation

Variables not associated with reactions (strong evidence, multivariate analysis)
- Predonation blood pressure

Table 5.3 Strategies to reduce reactions among young blood donors

Predonation education
Drive setup and environment
Staff supervision and phlebotomist skills
Selection criteria (e.g., estimated blood volume) for whole blood donors
Automated red cell collection
Interventions
- Water ingestion before donation and within 10–20 min of phlebotomy
- Distraction during phlebotomy
- Muscle tension during phlebotomy

Postreaction instructions to donors and parents

donation.[14] Many other variables related to the donor, environment, or staff have been reported to have weaker associations with immediate adverse reactions after blood donation, although the supporting data are generally weak and often not consistent among studies.[7] Finally, autologous blood donors might have significant medical conditions that potentially increases their risk for postdonation reactions compared to healthy, allogeneic blood donors.[17]

Most publications report on immediate donation-related reactions or reactions that occur soon after the blood donation, and less information is available on possible chronic complications. But in a recent study, allogeneic blood donation did not have a long-term effect on the risk of cardiac ischemia.[18] Germain *et al.* compared a group of 50,889 eligible blood donors who made 0.36 donations/year to 12,357 donors disqualified for false-positive infectious disease markers as a control group of healthy donors during a 17-year study in Canada.[18] There was no statistical difference in the incidence of hospitalizations or deaths attributable to coronary heart disease in the group of donors who remained eligible (3.60/1000 person-years) compared to the control group of disqualified donors (3.52/1000 person-years; rate ratio 1.02; 95% confidence interval, 0.92–1.13).

Preventing syncopal reactions at blood drives

Focusing on the factors that most strongly predict immediate and delayed reactions, blood centers have recently applied strategies to improve safety for the youngest and most susceptible group of blood donors. In 2008, an AABB Task Force recommended that blood centers adopt one or more of the measures in Table 5.3 to reduce reactions among young donors, and develop monitoring programs to continually assess donation safety.[19] Operational tactics to improve donation safety aim to recruit donors less likely to have reactions, to modify the drive environment, or to use automated (apheresis) procedures instead of whole blood collection. Physiologic strategies may reduce an individual blood donor's risk of a reaction, such as having the donor drink water shortly before the phlebotomy or perform applied muscle tension maneuvers.[20] Psychological aspects of the donation experience may be addressed by gauging donors' fear and anxiety, providing donors with information about coping strategies before blood donation, or distracting their attention during the phlebotomy. Each measure is supported to varying degrees by controlled trials or by observational data and predictive models.

In 2009, the American Red Cross and Blood Systems Inc. independently made operational changes in their standard practice in an effort to reduce syncopal reactions among young whole blood donors.[21,22] Both centers introduced precautionary measures, which included new donor selection criteria that required individuals to have an estimated blood volume greater than 3.5 L to prevent loss of more than 15% of their total blood volume with a standard whole blood donation. The AABB Standards define the minimum donor weight (50 kg or 110 lbs.) and maximum collection volume (10.5 mL/kg) in order to limit acute blood loss. These measures protect most but not all donors, especially young female donors who have an estimated blood volume less than 3.5 L based on the Nadler equation that takes into account sex, height, and weight. These young, female donors with low blood volume disproportionately account for the presyncopal reactions and syncope-related complications. The programs at both American Red Cross and BSI also addressed donor education, drive environment and supervision, predonation water, and muscle tension during collection.

In the American Red Cross, full implementation of the new donor selection criteria and other preventive measures resulted in a 33%, 25%, and 18% reduction in reactions among 16-, 17- and 18-year-old donors, respectively, compared to the baseline rates before the operational changes.[21] The benefit was most pronounced for the youngest, most susceptible donors. When the data were stratified for first-time donation status and sex, 16-year-old girls had the same relative risk of presyncopal reactions as their 19-year-old counterparts (Figure 5.1). Moreover, a significant 14% decrease in syncope was observed among 16-year-old donors with the new selection criteria. The rate of injuries at the collections sites was low in each year, but no consistent changes were observed over time for any age group. The most encouraging result, however, has been the sustained reduction in the overall reaction rates among 16–18-year-old donors observed over the subsequent two years, despite the dynamic nature of operations on high school drives, and the increased recruitment of young donors each year (Figure 5.1).[21]

Similarly, Tomasulo and colleagues at Blood Systems reported that using the new selection criteria to ensure an estimated blood volume of at least 3.5 L for 17- to 22-year-olds and other

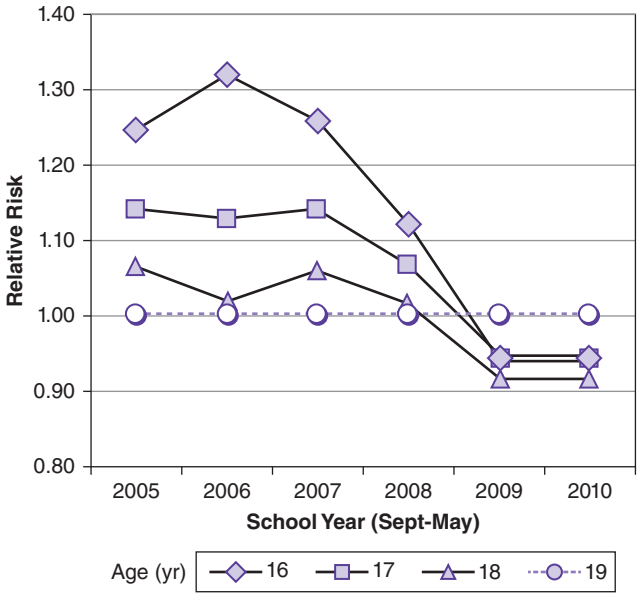

Figure 5.1 Relative risk of reactions among female, novice (first-time) whole blood donors by age (16 to 19 years), comprising different age groups (16 to 18 years compared to 19 years; relative risk = 1.0). Before implementing the safety measures (2005–2007), the overall rate of reactions was highest among 16-year-olds compared to their older peers. The new selection criteria for estimated blood volume became effective in the 2009 school year, and eliminated the excess risk for the young donors compared to 19-year-olds. The benefit has been sustained over 2 years.

interventions decreased the aggregate reaction rates in male and female donors by 24%.[22] In addition, the measures were associated with a 25% decrease in delayed reactions occurring more than four minutes after the phlebotomy was completed, and a 38% decrease in off-site reactions among female donors. Falls were infrequent in all donor groups, occurring at a rate of 1.4 per 1000 donations before the interventions. Multivariate analysis clearly showed that the interventions decreased the occurrence of reactions in susceptible groups, but the known risk factors (young age, first-time donation status) were still associated with relatively higher reaction rates than the comparison groups.[22]

These data from the American Red Cross and Blood Systems independently support the benefit of the selection criteria for estimated blood volume to mitigate reactions among young donors, largely to the extent predicted by statistical models.[21,22] In both reports, the aggregate effect of the selection criteria for estimated blood volume reduced reactions by at least 20% in susceptible groups. As expected, the measures did not eliminate the risk of reactions after whole blood donation. Both blood centers noted that the inconsistent or incomplete implementation of the provision of water and use of applied muscle tension likely limited the effectiveness of these interventions. Both studies also documented a low rate of falls or injuries at the collection sites in all donor groups, which did not change after introducing the operational changes. These studies were large enough to detect a 20% reduction in these uncommon events but may have missed a smaller difference. Alternate approaches to predict and prevent presyncopal reactions that show promise in small trials evaluate collecting smaller volumes (350 mL rather than 450 mL) from young (16- to 18-year-old) first-time donors, screening to identify and educate fearful donors, and predonation measurements of hemodynamic responses to standing before and after water ingestion.[23–26] Unfortunately, no single preventive measure has been shown to have a significant effect on the rare but potentially serious injuries resulting from syncope after whole blood donation. Future operational efforts and research studies should evaluate other possible evidence-based approaches to reduce the risk of syncope after whole blood donation.

Phlebotomy-related complications

Whole blood and apheresis collections utilize large-bore (16-gauge needles) for phlebotomy, which achieve rapid blood flow and minimize clotting and hemolysis but also introduce a risk of injury.[27] General guidelines for phlebotomy emphasize the importance of staff training, technique, and experience, as well as knowledge of the anatomy of the antecubital area and careful selection of the vein.[28] The median cubital vein, in the center of the antecubital space near the elbow crease, is often prominent and well-anchored lying between muscles, making it a good first choice for phlebotomy. The basilic vein, located on the medial aspect (i.e., closest to the body) of the forearm, runs close to the brachial artery and nerve and is less likely to be visible or palpable, which increases the risk of nerve injury or arterial puncture. Although these generalizations are useful guides, the anatomic relationships are more complex and variable than depicted in textbooks.[29] Cutaneous nerve branches are closely associated with veins and anatomic variation is common, making it impossible to avoid them completely during phlebotomy. Needle adjustment after insertion should be limited to no more than one forward-to-backward maneuver, with careful attention to the donor's comfort level. The phlebotomy should be discontinued if the donor cannot tolerate the venipuncture, and the needle should be removed if it is readily apparent that the venipuncture was not successful. Despite adherence to these general guidelines and good technique, phlebotomy as with any invasive procedure carries with it inherent risk that cannot be completely eliminated.

Adverse reactions such as sore arms, hematomas, or bruises are relatively common but usually self-limiting; rarely, more severe nerve injuries or chronic complications occur after phlebotomy. Hematomas are raised areas of localized swelling, and bruises (contusions) are flat areas of discoloration. Both result from local injury and blood leaking from the punctured blood vessel that accumulates in the subcutaneous tissue and spreads along fascial planes. As the swelling subsides, the extravasated blood is broken down and reabsorbed, and the area can change in shape, size, and discoloration over a spectrum of blue, black, purple, yellow, and green before resolving completely within a few weeks.

Hematomas or bruises may be noted by the staff at the collection site, but more often develop after the donor leaves the drive, complicating about 1% of whole blood and 3.5% of apheresis donations (Table 5.1). When donors were questioned about bruises or other symptoms several weeks after a donation, additional information was elicited about minor complications and the rate of bruises reached 8% to 22%.[8,9] Although this suggests that some minor reactions are not routinely captured, the available blood center data still serve as a relevant gauge of the more severe or concerning reactions, and identify hematomas that cause such discomfort, pain, or distress that about 1 in 10,000 whole blood and apheresis blood donors seek outside medical care (Table 5.1).

Hematomas may be alarming to donors, especially if they extend along the arm or progress through the various stages of discoloration, but typically resolve completely within a few weeks. Treatment of the area with ice immediately and intermittently for

24 hours after the phlebotomy, followed by warm compresses or soaks, may provide symptomatic relief and promote healing. Over-the-counter medications such as acetaminophen may also be taken as directed for pain. In very rare cases, the phlebotomy site may show signs of infection or septic phlebitis (e.g., redness, swelling, or "red streaks" extending up the arm), which may be treated with antibiotics or resolve on its own. In case reports, superficial venous thrombosis in the upper extremity has occurred after blood donation, although a causal relationship with the donation may not be clearly established.

Arterial puncture is suspected in about 1.4 in 10,000 whole blood donations (Table 5.1). Signs of an arterial puncture are rapid filling of the collection bag, often within 3 minutes; bright red blood; and pulsatile flow. All of these signs might not be present, making some arterial sticks difficult to distinguish from venous draws. Possible arterial punctures are more often reported in young, male donors, but can occur in other donors because of anatomical variation in the location of the artery in the forearm. Whenever a possible arterial puncture is suspected, staff should discontinue the collection, remove the needle, and apply direct pressure to the site for 10 minutes or more, until bleeding has stopped. Donors typically recover without rebleeding or further consequences, but about 7% of donors with a suspected arterial puncture sought additional medical care, likely related to the resultant hematoma or bruise. In extremely rare cases, an arterial puncture has resulted in complications that required surgical intervention, such as pseudoaneurysm (three published reports), arteriovenous fistula (four published reports), or compartment syndrome (two published reports), after whole blood donation.[27]

Phlebotomy-related nerve injury

Phlebotomy-related nerve injuries are uncommon and typically resolve without sequelae within weeks, but in rare cases have long-term debilitating consequences. Most patients experience characteristic symptoms when the needle is advanced in the arm suggestive of direct nerve trauma, often described as sharp, shooting pain or tingling that radiates to the hands or fingers. Donors may also report immediate numbness or tingling that persists after removing the needle or intense pain with burning, lancinating, or electrical sensations. The relationship of hematoma formation to the development of nerve damage and symptoms is unclear, and most hematomas do not cause symptoms suggestive of nerve irritation. However, hematomas may aggravate nerve dysfunction and pain possibly by impinging on damaged nerves or traumatized areas.[29,30]

Based on hemovigilance data from the American Red Cross, suspected nerve injuries occurred in about 3 in 10,000 whole blood donors; of those, 13% sought additional medical care after the event (Table 5.1). Almost all (>90%) donors who report symptoms of nerve injuries will recover completely within three months.[30] Some donors may report mild residual numbness that persists over an extended duration. Permanent nerve injury after phlebotomy is an extremely uncommon but potentially debilitating outcome, and reliable estimates on its incidence are not available because it is so infrequently encountered among blood donors. Given the close association of nerves with veins and the unavoidable possibility of direct injury to nerves with phlebotomy, factors other than direct nerve contact by the needle appear to be necessary for the chronic pain syndrome to occur. Chronic regional pain syndrome type 2 (CRPS II, previously called causalgia or reflex sympathetic dystrophy) describes neuropathic pain after confirmed nerve injuries. CRPS symptoms vary in severity and duration, but most cases are mild and individuals recover gradually with time. In more severe cases, individuals may have long-term disability. Treatment options for CRPS neuropathic pain include rehabilitation and occupational therapy, psychotherapy to treat associated depression and psychological symptoms, medications, sympathetic nerve block, and surgical sympathectomy. No drugs have yet been approved by the US Food and Drug Administration (FDA) to treat CRPS, but several different classes of drugs such as nonsteroidal anti-inflammatory drugs, corticosteroids, opioids, and botulinum toxin injections have been used to effectively treat some patients, especially early in the course of disease.

Reactions after automated collection of blood components

Advances in apheresis (automated) technology now allow simultaneous collection of multiple standard blood components during a single procedure, yielding various combinations of plasma and blood components such as two units of red blood cells (double red cell collection [2RBC]) or two or three units of platelets (double or triple plateletpheresis), often with a concurrent unit of plasma from one donation. Blood centers have increasingly relied on apheresis donors to optimize the collection process, and increase operational efficiency and cost-effectiveness. Continuing on an upward trend in recent years, apheresis platelets accounted for over 90% of the platelet units produced in the United States in 2011.[1] Triple plateletpheresis contributes substantially to that increase, accounting for about 15% of the apheresis platelet units distributed by the American Red Cross. Similarly, 2RBC collections continue to increase each year, now accounting for 12.6% of the total US supply of red cell units in 2011.[1]

As with whole blood donation, automated (apheresis) procedures are generally well tolerated, but some donors will experience phlebotomy-related or syncopal complications (Table 5.1). In addition, apheresis complications may result specifically from the device or anticoagulant (e.g., citrate reactions), preparatory regimens (e.g., G-CSF and/or corticosteroids for neutrophil collection), or frequency of procedures. The available data from several different blood centers, however, suggest that automated collections have a favorable safety profile compared to whole blood donation.[31,32] Hematomas are the most frequent complication and are more commonly reported after apheresis than WB donation because automated collections often use both arms for venous access (Table 5.1). The overall rate of minor complications at the collection site is higher for automated procedures than WB collections, reflecting primarily minor citrate reactions and small hematomas. Automated collection procedures had lower rates of presyncope and syncope than whole blood donation, which likely reflects donor demographics, more stringent selection criteria, and the use of saline replacement with automated procedures.

Medically serious complications are less likely after apheresis than WB collection, with observed rates of major complications at the collection site of 7.4, 5.2, and 3.3 per 10,000 donations for WB, plateletpheresis, and automated red cell procedures, respectively.[2] Similar rates of reactions requiring additional medical care occurred after WB donation (3.2 per 10,000) compared to automated procedures (2.9 per 10,000).[2] Hospitalization after donation was reported for 46 whole blood donors (1 in 130,749 donations) and eight apheresis donors (1 in 84,722 donations); a causal relationship between the donation and the hospitalization was not established in all cases.

As observed for whole blood donation, multivariate analysis reveals that young age, first-time donation status, female gender,

and low weight are independently associated with the risk of reactions after automated red cell collection and plateletpheresis. Yuan *et al.* reported moderate to severe adverse events in 47 per 10,000 plateletpheresis or automated red cell collections over a 2-year period in a hospital-based donor center, and found that small, female donors with lower predonation hematocrit were at higher risk of moderate to severe vasovagal-type reactions than other donors, especially when RBCs were collected.[33] Reactions had a similar dampening effect on return donation by first-time apheresis donors comparable to that observed for whole blood donors. Among experienced donors, however, reactions had less of an effect on retention and decreased the rate of return by about 28% for whole blood donors but only about 4% for 2RBC donors.[6]

Citrate reactions and other immediate complications during apheresis procedures

Citrate is used as an anticoagulant during apheresis procedures because it effectively chelates divalent cations such as calcium to transiently and immediately inhibit the coagulation cascade. Citrate causes minimal side effects in donor plasmapheresis because the citrate is mostly in the retained plasma. Plateletpheresis, large-volume leukapheresis, and hematopoietic progenitor cell collection are more likely to expose the donor to the effects of citrate toxicity. Greater exposure to citrate during triple plateletpheresis was associated with an increase in mild citrate reactions compared to double plateletpheresis, but did not substantially affect donor safety or product quality in one study.[34] Symptoms are usually transient and rapidly reversible because of citrate metabolism occurs within minutes in the liver, kidneys, and muscles. In addition, release of parathyroid hormone mobilizes calcium from body stores and increases its absorption from the kidney to restore calcium hemostasis. Despite these compensatory mechanisms, citrate infusion can acutely decrease the concentration of ionized calcium to cause symptoms such as perioral tingling and paresthesias, chills, nausea, twitching, and tremors during the apheresis procedure. If severe, citrate toxicity can progress to carpopedal spasm, seizures, tetany, and cardiac arrhythmia.

Prompt attention to mild symptoms usually requires only pausing the procedure, slowing the re-infusion rate, or decreasing the amount of citrate infused by increasing the whole blood-to-citrate ratio and allowing for dilution and clearance of citrate. In addition, donors may be given oral calcium in the form of calcium-containing antacids (e.g., Tums), or the procedure may be stopped if symptoms persist or worsen. Intravenous calcium is rarely if ever needed to reverse the citrate effect during donor apheresis procedures and should not be used in routine practice. Donors who have had severe or unusual citrate reactions during automated procedures should be evaluated for possible underlying factors or medications such as loop diuretics that could predispose to these adverse events. The propensity for citrate reactions depends not only on donor characteristics, but also on device-related factors, such as the citrate infusion rate or extracorporeal volume of the device.

Oral calcium supplementation during automated collection procedures may reduce the severity of parasthesias and improve ionized and total calcium levels.[35] However, multivariate analysis revealed that oral administration of calcium was not associated with a reduction in overall symptom development and did not prevent the occurrence of more severe symptoms during donor apheresis procedures.[35] The possible significance of long-term metabolic effects of repeated citrate exposure on bone mobilization and calcium metabolism are not well characterized and remain an area of study.

Equipment and disposables used in apheresis collections may cause unusual reactions in rare cases, such as allergic reactions among repeat donors linked to plateletpheresis collection sets sterilized with ethylene oxide.[31,32] Preventive measures include avoiding use of certain disposables if sensitization is suspected, or minimizing exposure to ethylene oxide by repeatedly priming the disposable collection set or using kits closest to their expiration dates. Air embolism is a very rare complication of apheresis procedures because the instruments have sensors to detect air within the extravascular circuit that stop the procedure. But symptoms of air embolism are still possible if more than 3–8 mL/kg of air enters the donor's venous system through either a leak in the access, instrument failure, or operator's error. These symptoms of air embolism include dyspnea, tachypnea, cyanosis, tachycardia, and hypotension as air enters the right ventricle and pulmonary artery with obstruction of the right ventricular output and pulmonary artery vasoconstriction. If collection staff expect air embolism, they should stop the procedure and place the donor in the Trendelenberg position (i.e., lay the donor on their back and raise their feet higher than their head) on their left side. If the air does not dissipate or symptoms worsen, surgical intervention to aspirate the air through a pulmonary artery catheter may be necessary.

Procedure-related complications related to donation frequency or multiple component collection

The high volume and efficiency of apheresis collection procedures, as well as the frequency and allowable interval for repeat donations, pose potential acute and long-term risks to donors, such as cellular depletion, iron depletion, and serum protein loss. The current regulations that govern donor selection define precautions for adequate pre- and postprocedure cell counts and serum protein values before donation, as well as limits on the donation interval and frequency for apheresis procedures.

After plateletpheresis, a donor's platelet count may decrease by 20–30% but quickly returns to baseline within about four days.[31] Current FDA-approved apheresis devices have different methods to ensure that the donor's platelet count remains above a predefined set value at the completion of the procedure, typically 100 platelet/μL, which have been validated in practice by blood centers. An intensive schedule of serial plateletpheresis procedures (e.g., 5–15 procedures within 10–30 days) was associated with only transient decreases in platelet counts, which in some cases rebounded to above baseline values about a week after the final procedure. The transient platelet count decrease and recovery after serial collection procedures are generally larger and last longer for female donors than male donors, but changes are transient and recovery occurred promptly after donation.

Recently, evidence of progressive decreases in platelet counts after years of donation in some individuals raised concerns about possible long-term effects of frequent apheresis platelet donation.[36–38] Lazarus *et al.* retrospectively examined platelet counts from 939 individuals who had 11,464 plateletpheresis donations over four years, and described sustained decreases in platelet counts in some donors that correlated with donation frequency. However, these observations were not observed by other large blood centers. A retrospective review of plateletpheresis records at a regional blood center revealed no clinically important decrease in platelet counts among individuals donating multiple platelet components up to 24 times per year, regardless of interdonation interval.[38] These observations were confirmed by several other facilities, and the available data do not demonstrate clinically important changes in

platelet or lymphocyte counts in frequent plateletpheresis donors. Plateletpheresis procedures collect negligible red cells in the component, but frequent plateletpheresis donors lose as much as 80–100 mL of whole blood with each donation as a result of samples taken for infectious disease testing and residual red cell loss in the collection sets. Chronic, small-volume red cell losses sufficient to cause iron depletion have been described in case reports of frequent plateletpheresis donors.

Several studies have examined the long-term effects of intensive plasmapheresis regimens on donor serum protein, albumin, and immunoglobulin levels. Serial plasmapheresis donors can give 625–800 mL of plasma twice weekly in the United States, provided they meet all selection criteria. Serum protein, albumin, and IgG concentration are statistically lower in frequent plasmapheresis donors compared to nondonors, but their levels do not correlate with the intensity of donation and remain stable over time in most donors.[39,40] Moreover, plasmapheresis donors who gave up to 45 L of plasma per year did not develop impaired humoral and cellular immunity, maintained adequate iron stores, and did not show signs of increased cardiovascular risk by biochemical measures.[39] Only 4–16% of regular donors discontinued plasma donation when IgG, total serum protein, or hemoglobin fell below acceptable values; most donors stopped for socioeconomic or medical reasons unrelated to plasma donation.[40] Taken together, these studies support the safety of long-term intensive donor plasmapheresis under the current regulations, and provide some reassurance about the reasons that donors discontinue participation in the programs. Finally, none of the 12 deaths following source plasma donation that were reported to the FDA in 2005–2006 were determined to have a causal relationship with the plasmapheresis procedure.[41]

Special considerations: granulocyte collection

Granulocyte collection (leukapheresis) poses unique risks for donor complications.[1] To collect sufficient numbers of granulocytes for an adequate therapeutic dose, healthy donors are given corticosteroids (e.g., dexamethasone) and/or granulocyte colony-stimulating factor (G-CSF) prior to the leukapheresis procedure. G-CSF may cause short-term side effects such as bone pain, myalgia, and headache in granulocyte donors. The rare complication of splenic rupture seen with hematopoietic progenitor cell collection has not been reported in G-CSF-stimulated granulocyte donors, likely because of the lower dose and shorter course of treatment. To date, the available data support the use of G-CSF stimulation in volunteer donors and have not detected long-term cardiac, inflammatory, or malignant consequences among granulocyte donors who received G-CSF on three or more occasions.[42]

The risk of subcapsular cataracts in granulocyte donors who received corticosteroids has been evaluated in two controlled studies. Differences between the treatment and the control (unstimulated) groups were not significant, but the tendency for bilateral occurrence of cataracts was observed exclusively among glucocorticoid-stimulated granulocyte donors. The relatively small size of both studies suggests that ongoing surveillance is prudent to better define the prevalence of posterior subcapsular cataracts in this donor population.[43,44]

Iron deficiency after blood donation

The impact of blood donation on depleting donor iron stores has been recognized for well over 30 years.[45,46] With each whole blood collection of 500 ml, an average of 200–250 mg of iron from

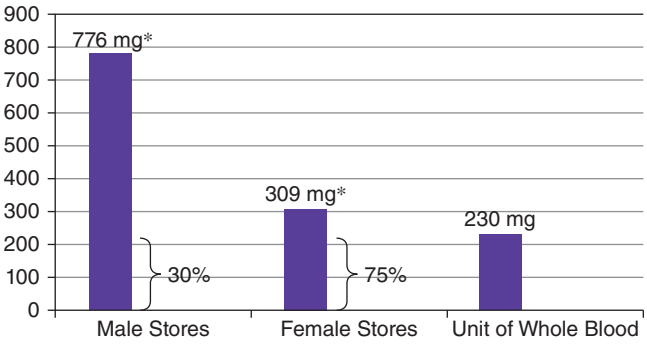

Figure 5.2 Iron stores versus iron removed from donation. Source: Cook *et al.* (2003).[53] Reproduced with permission of the American Society of Hematology.

RBC hemoglobin is removed. This amount represents about 25% of the average iron stores in men but almost 75% of the iron stores in women (Figure 5.2). The acute iron loss is replenished by gastrointestinal (GI) absorption of dietary iron, which occurs gradually over many months. However, individuals may donate blood as frequently as every 56 days (in North America), an insufficient time for a donor to replace the iron that is lost. The cumulative effects of repetitive blood donation at shorter intervals than iron can be replenished eventually results in a deficit in body iron that is dependent primarily on donation intensity and gender, as well as other factors to be discussed in this chapter. In addition, the consequences of iron depletion in terms of donor well-being and potential strategies to manage donor iron loss, including extending the minimum required donation interval, decreasing donation frequency, testing of iron status, and iron supplementation, will be considered.

Brief review of iron physiology

Iron is an essential element involved in several key physiologic processes. In association with heme, it facilitates the reversible binding of oxygen by red blood cells and the binding of oxygen to muscle myoglobin and mitochondrial cytochromes. Nonheme iron is involved in the actions of a number of cellular enzymes. Dietary iron is absorbed by enterocytes in the duodenum and proximal jejunum. Absorption is tightly regulated because iron in excessive amounts is toxic to cells (e.g., by generation of ferric peroxides and free radicals) and there is no defined mechanism of excretion. Iron from animal sources (heme iron) is more bioavailable (~35% absorbed) than nonheme iron (vegetable sources: ~10% absorbed).[47] Men normally absorb ~1 mg/day, equaling basal losses from the GI tract and skin. Iron absorption in premenopausal women is ~0.5 mg/day greater, because of additional losses from menstruation. Absorption capacity increases in proportion to the level of iron deficiency (Figure 5.3), reaching a maximum that averages 4–5 mg/day in frequent donors,[48,70] and is enhanced with supplemental iron.[49]

Hepcidin has been recognized as the master regulator of iron homeostasis. This 25-amino-acid peptide hormone produced in the liver controls iron absorption and release of iron from storage sites through inhibition of the transmembrane iron receptor, ferroportin.[50,51] In iron deficiency, hepcidin decreases, permitting ferroportin to shuttle iron into enterocytes from the intestinal lumen and out of hepatic and other storage sites, to be transported by transferrin for cellular uptake via transferrin receptors located on early erythroid and, to a lesser extent, other nucleated cells. Iron not

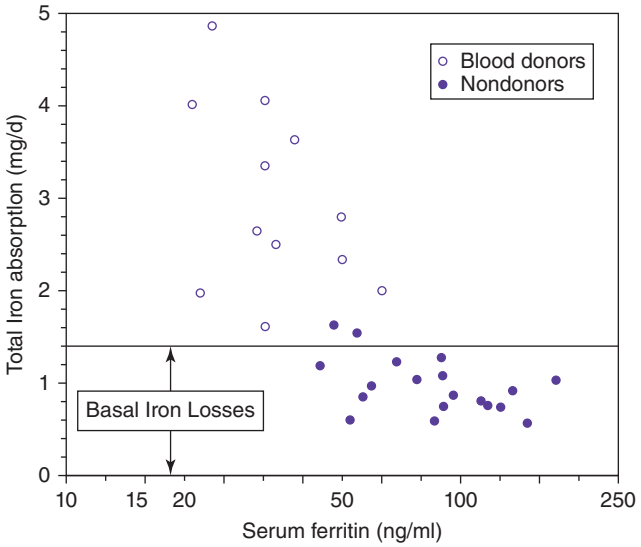

Figure 5.3 Iron absorption in blood donors. Modified from Hallberg *et al.* (1997).[47] Reproduced with permission of American Society for Nutrition.

directly utilized in physiologic pathways is stored in tissues as ferritin; small amounts present in blood are in equilibrium with tissue ferritin, which is considered a reliable indicator of available storage iron, especially in healthy blood donors, who have low rates of inflammatory conditions that can result in elevated ferritin values irrespective of iron status.[52] The total body iron content in men averages approximately 50 mg/kg (~4000 mg), whereas women have 35 mg/kg (~2500 mg). The majority of the total (70–80%) exists in red blood cells bound to hemoglobin. Approximately 10% of body iron is engaged in other cellular physiologic functions, such as enzymes and cytochromes, or bound to transferrin. Only 10–20% of the total is present in the form of tissue iron stores that are available for erythropoiesis and metabolic use. Cook *et al.* estimated tissue iron stores of 776 ± 313 mg in men and 309 ± 346 mg in women.[53] The loss of approximately 230 mg iron with each whole blood donation along with the limited stores and capacity for absorption lead to a high incidence of iron deficiency in frequent donors, especially women.

Measurement of iron status

The only point-of-care screening test currently used to qualify a blood donor is the capillary (fingerstick) hemoglobin ≥12.5 g/dL in both women and men in the United States and Canada. Some countries have adopted a higher standard in men (e.g., 13.0 or 13.5 gm/dl) that reflects the higher range of normal for hemoglobin.[54] The minimum hemoglobin threshold is intended to prevent collection of blood from donors with anemia (this is approximate, because according to WHO the lower range of normal hemoglobin in women is 12.0, and the lower range for men is 13.0 gm/dl), but does not prevent collection of blood from donors who are iron deficient. Various laboratory tests have been used to assess the iron status of blood donors and are listed in Table 5.4.

Serum (or plasma) ferritin

Ferritin, a protein that stores excess iron and releases it in a controlled way, reflects the level of tissue iron stores and can be assayed in either serum or plasma. Using EDTA (ethylenediaminetetraacetic acid) plasma, ferritin concentration is ~5% lower than serum.[55] Blood levels are believed to result from passive equilibration from cell or tissue sites. Each ng/ml of ferritin in blood equals 8–10 mg of iron in the storage compartment.[56,57] Normal population values differ by gender and age, with adult males age 20–60 years reaching a plateau between 134 and 150 ng/ml, and females 20–59 years between 32 and 53 ng/ml.[58] Ferritin increases in women after menopause, so that after age 60 the average is 86 ng/ml. The "classic" cutoff value, 12 ng/ml, has been utilized as a specific but insensitive indicator of absent iron stores in the clinical setting[59] and in blood donors.[60] One study found that this cutoff failed to identify iron depletion in over one-third of cases in blood donors.[61] Two clinical studies based largely on bone marrow staining for iron have found 15 ng/ml to more accurately reflect iron deficiency anemia.[62,63] Studies based on increased levels of soluble transferrin receptor (sTfR), a truncated form of the transferrin receptor that is released into blood when early erythroid cells become deprived of iron, suggest a ferritin concentration of 22–40 ng/ml is more sensitive as an indication of iron-deficient erythropoiesis.[64,65]

The classic description by Finch correlated a drop in ferritin levels with blood donation activity, showing that a ferritin level of less than 12 ng/mL increased with the number of donations in the previous year.[45] Simon *et al.*, in an observational study of blood donors, showed that the frequency of iron depletion as measured by serum ferritin ≤12 ng/ml was zero in male first-time donors but 12% in female first-time donors, reflecting the impact of menstrual blood loss.[46] The frequency in men increased to 2% and in women increased to 20% with three blood donations in one year. A direct effect of donation intensity on iron stores has been shown in multiple studies, which indicate a stronger effect of donation frequency (i.e., donations/year) rather than lifetime donations (Figure 5.4).[66,67]

Table 5.4 Assessment of Iron Status in Blood Donors

- Ferritin
- Soluble transferrin receptor (sTfR)
- Soluble transferrin receptor/ferritin ratio
- Zinc (Free Erythrocyte) Protoporphyrin (ZPP)
- Red blood cell parameters (HYPOm, CHr)

HYPOm % hypochromic mature RBC; CHr, hemoglobin content of reticulocytes.

Soluble transferrin receptor

The highest density of transferrin receptors exists on the surface of erythroid cells. A reduction in iron availability leads to increased TfR synthesis and shedding of soluble transferrin receptors (hence sTfR) into the blood. Levels greater than the normal reference range (95% CI) suggest tissue iron deficiency in the absence of conditions that are also known to increase sTfR levels, including accelerated erythropoietic proliferative activity (e.g., hemolytic anemia and thalassemia), race (~9% higher in black subjects), and altitude (also ~9% higher).[56] Because of the lack of a reference standard for transferrin, the results vary according to the assay used. However, research studies indicate sTfR levels reflect the functional iron compartment and correlate with depleted iron stores in bone marrow preparations[59] and the response to oral iron therapy in otherwise healthy females with anemia.[68]

As iron depletion progresses to the stage of tissue iron deficiency, sTfR continues to increase as ferritin levels reach the lower limit of

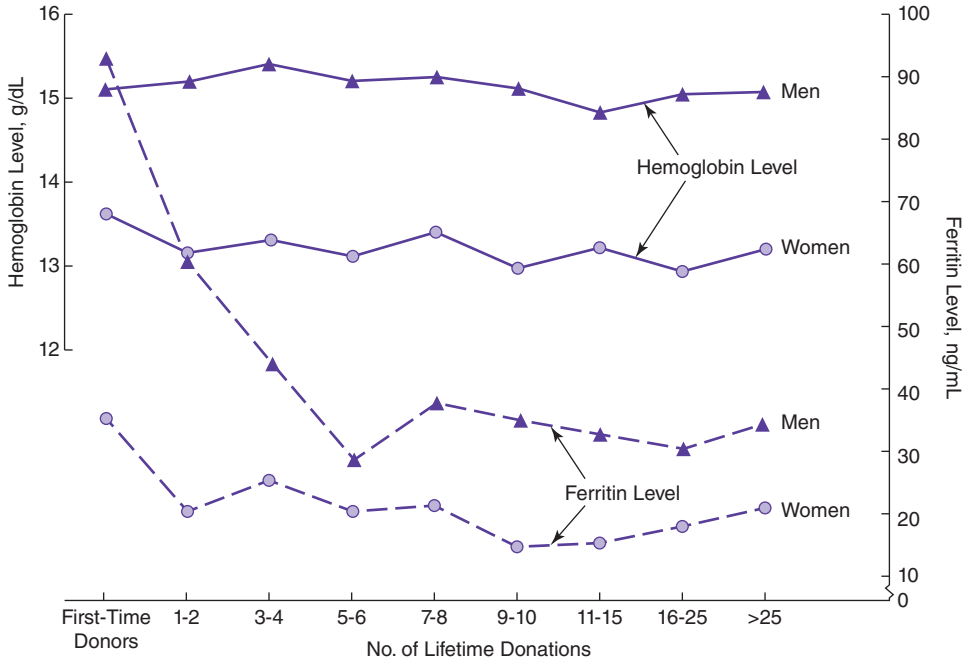

Figure 5.4 Changes in hemoglobin and ferritin with successive blood donations.[46]

detection. sTfR values (representing functional iron deficiency) have been combined with ferritin measurements (representing storage iron levels) into a ratio, log sTfR/ferritin.[61,69,71] A sTfR/log ferritin "index" has also been utilized to distinguish iron-deficient erythropoiesis from storage iron depletion.[59,68] However, an advantage to using the log sTfR/ferritin construct was demonstrated by Skikne and Cook,[53,64] who were able to assess iron stores quantitatively by performing careful measurements of iron loss in serial phlebotomy subjects bled until they became iron depleted, as expressed by the formula:

$$\text{Body iron(mg/kg)} = -[\log(\text{sTfR/Ferritin}) - 2.8229]/0.1207$$

This methodology allows an estimation of tissue iron stores, which can be uniquely expressed as the iron surplus in stores (positive values) or the iron deficit (negative values) in tissues, and also permits an estimation of iron absorption in blood donors in longitudinal studies.[49,70]

In the REDS-II Donor Iron Status Evaluation (RISE) study (described in the "Other Measures" section), sTfR did not correlate with iron-deficient erythropoiesis (IDE) as well as plasma ferritin, R^2 0.54 versus R^2 −0.96.[69] A log sTfR/ferritin value of 2.07 (97.5% of the upper limit of the reference range) equated to a ferritin level of 26.7 µg/L by multivariate regression, suggesting this ferritin level reflected iron-deficient erythropoiesis in healthy blood donors. At this threshold, ferritin had 95.1% sensitivity and 89.6% specificity in identifying IDE, and sTfR added little additional diagnostic information.

Other measures

A limitation with ferritin testing in blood donors is that results may not be available for several days. A point-of-care test using capillary (fingerstick) samples is being evaluated to assess blood donor iron status. Zinc protoporphyrin (ZnPP), also called free erythrocyte protoporphyrin (FEP), can be measured in capillary samples using a portable hematofluorometer. During the last step of porphyrin ring synthesis, zinc is chelated by protoporphyrin IX if iron is limited, resulting in increased levels of erythrocyte ZnPP–mole Heme (ferrous protoporphyrin).[72] Lead intoxication and thalassemia, conditions that have a low prevalence in blood donors, also result in elevated values.[73] A study of over 5000 accepted blood donors found that 6.9% of male donors and 9.8% of female donors had subclinical iron deficiency as defined by ZnPP levels of ≥100 *m*mol/molheme.[74] Some studies show early detection of iron deficiency and correlation with hemoglobin deferral in blood donors. One trial showed that a positive predictive value of ZnPP in predicting deferral of the donor after one or two donations was 75%, and a serum ferritin concentration ≤12 µg/L of only 26%.[75] Another trial reported that elevated ZnPP levels (using venous, not capillary, blood) aided in the prediction of subsequent hemoglobin deferral when added to other variables, including previous hemoglobin value, age, gender, time since previous visit, and total number of blood donations over two years.[74] However, an attempt to validate this model in another donor population using different hemoglobin eligibility criteria was unsuccessful.[76]

Changes in conventional red blood cell morphologic parameters, including mean corpuscular volume (MCV), mean cell hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), occur late in the development of iron depletion and are insensitive, resulting in low correlation with reduced iron levels (R^2 minus 0.08–0.17).[78] RBC parameters including MCHC (<330 g/l; $R^2 = 0.12$) and MCV (<80 fl; $R^2 = 0.00$) were inferior to hemoglobin ($R^2 = 0.63$) in predicting subsequent hemoglobin deferral in another study.[79]

Although not a point-of-care test, measurement of RBC indices by laser light scatter using specialized hematology analyzers (ADVIA 120, Siemens Healthcare Diagnostics, Deerfield, IL, USA; and Sysmex XE-5000, Kobe, Japan), with "same-day" results available within hours, has also been evaluated in blood donors.[77,80] Selected RBC subpopulations have been found to be more sensitive indicators of functional iron deficiency than biochemical iron tests

in renal failure patients who are treated with erythroid stimulating agents, in pregnancy, and in pediatric patients with iron deficiency.[80,81–83] In young women with iron deficiency anemia, the percentage of hypochromic mature red blood cells (%HYPOm) was found to correlate with sTfR (area under the receiver operating characteristic [ROC] curve, or AUC, of −0.98) and returned to normal after oral iron therapy.[81] CHr (cellular hemoglobin content in reticulocytes) measures incorporation of iron into developing reticulocytes that can be detected within their three-day lifespan.[84] %HYPOm is a time-averaged indicator of iron that is incorporated within the three-month lifespan of mature RBCs. These parameters are analogous to monitoring glucose levels and HbA1c levels in diabetics, respectively. Using the ADVIA 120 analyzer, one study in blood donors found adequate sensitivity of CHr at 32 pg cutoff and HYPOm at 0.3% cutoff individually (57.5% for both measures) and combined (69%) in donors with iron-deficient erythropoiesis, along with excellent specificity (~90%).[61] A smaller study found 81% sensitivity and 89% specificity for a HYPOm equivalent parameter, RBC-Y, and lower sensitivity (69%) versus 93% specificity for CHr at 28 pg level.[71] Each study used the log [sTfR/ferritin] ratio as the "gold standard" to identify iron deficiency. Despite logistical issues, the investigators felt that RBC indices were superior to hemoglobin in the assessment of iron status blood donors.

In the multicenter RISE study, plasma ferritin and sTfR were evaluated in relation to RBC indices, including CHr and the % HYPOm red cells, to characterize absent iron stores (AIS) and IDE in blood donors.[69] The RBC index that performed the best overall was %HYPOm. At a %HYPOm cutoff value of 0.55%, sensitivity/specificity was 85%/57% for AIS and 72%/68% for IDE. CHr had lower diagnostic usefulness than HYPOm or other indices. The RBC assays were better (greater AUC) at identifying more severe iron depletion (i.e., ferritin < 12 ng/ml). In a single-center study in blood donors, CHr using a cutoff value of 28 pg was reported to have excellent specificity for AIS (defined by sTfR/log ferritin >97.5th percentile, or 1.5); however, CHr was not sensitive for detecting "latent" iron-depleted donors.[85] An important limitation in using RBC indices is the manufacturer recommendation for testing within six hours of draw because cell swelling may affect the accuracy of the parameters, including HYPOm, hypochromic red blood cells (HYPOr), cell hemoglobin concentration mean (CHCM), and MCV but not CHr.[69,77]

The RISE study and the other studies summarized above found that RBC indices had only a modest value in assessing the iron status of blood donors. Ferritin provided the most useful laboratory information overall. In the two largest studies, a plasma ferritin value of 26.7 μg/L[69] in one and a serum value of 22 ng/ml[61] in the other provided the optimum discrimination between iron-depleted and iron-replete blood donors, regardless of gender.

Given the central role it plays in regulating iron homeostasis, serum hepcidin levels have also been evaluated in selected populations of blood donors. In non-anemic premenopausal women donors, hepcidin was found to correlate closely with ferritin levels (coefficient 0.66, $p < 0.001$).[86] As a diagnostic test for iron deficiency (defined as ferritin <15 ng/ml), hepcidin compared similarly to other biomarkers such as sTfR, and had an AUC^{ROC} of 0.87 (95% CI 0.82, 0.92). The reference range in non-iron-depleted women was 8.2–199.7 ng/ml. Using ROC analysis, a hepcidin level of 8 ng/ml had a sensitivity of 41.5% and high specificity of 97.6%; at a cut point of 18 ng/ml, sensitivity increased to 79.2% while maintaining specificity of 85.6%. In a longitudinal follow-up study, low baseline hemoglobin (AUC^{ROC} 0.88), ferritin (AUC^{ROC} 0.86), and hepcidin (AUC^{ROC} 0.81) values were predictive of future low hemoglobin deferrals.[86] Hepcidin and ferritin levels were also reported to be highly correlated in a study of first-time female donors and frequent male donors (Spearman r^2 0.74), and the predicted hemoglobin decline between donations varied according to hepcidin and ferritin. Hemoglobin was found to be 0.51 g/dL lower for subjects with low (≤45.7 ng/mL) or decreasing hepcidin and low ferritin (≤26 ng/mL), and hemoglobin was stable in donors with high (>45.7 ng/mL) or increasing hepcidin and low ferritin (≤26 ng/mL) levels ($p < 0.001$).[87] Based on these findings, hepcidin may provide a useful diagnostic tool in addition to ferritin to assess blood donor iron status.

Prevalence and risk factors for iron deficiency in blood donors

Iron depletion begins with the gradual loss of storage iron, a relatively small compartment as indicated above. Once stores are exhausted, a phase of IDE ensues, which then progresses to iron-deficiency anemia (IDA).[68] Iron deficiency is prevalent in the US population as a whole, especially among premenopausal women, in whom survey data reveal ferritin values <15 ng/ml in 14% of women aged 12–49.[58] In first-time blood donors, iron deficiency (defined as ferritin ≤12 ng/ml) is rare in men (under 1%) and reported in 6.6–12% overall.[46,66] Nearly 70% of the 9 million blood donors who donate annually in the United States are repeat donors, who are especially subject to iron depletion.[1] The prevalence of iron deficiency (using ferritin ≤12 ng/ml) reported in repeat donors ranges from 6 to 16% in men and from 28 to 63% in women.[60,66] Overall, using the most sensitive laboratory measures, iron depletion (defined as iron levels associated with impaired erythropoiesis; see below) is estimated to affect as much as 25–35% of the entire donor population.[88,89] Even individuals who exclusively donate by plateletpheresis may develop low iron because of the increased frequency allowed and fixed red blood cell losses occurring with each procedure (50–80 ml in samples and tubing).[90]

Low hemoglobin, a late consequence of iron deficiency, is the most common reason for donor deferral, with nearly 7% of presenting donors not allowed to donate because they cannot meet the minimum capillary hemoglobin standard of 12.5 gm/dl.[1] Hemoglobin deferral disproportionately affects women, with 17.7% of presenting women and 1.6% of men deferred.[91] Using the WHO defined thresholds of 13 gm/dl in men and 12 gm/dl in women, by definition, all men who are deferred are anemic, as are those women with hemoglobin values below 12 mg/dl. Prevalence estimates of iron depletion are high in hemoglobin-deferred donors. In one study, 53% of women and 61% of the men had iron measurements below lower gender-based limits.[92] The prevalence of iron depletion in controls (nondeferred donors) was also quite high in the study, reflecting the poor correlation of hemoglobin with iron status. Consistent with these findings, an Australian study of premenopausal women found mean ferritin to be lower in hemoglobin-deferred donors, 8.4 ng/ml, versus 27 ng/ml in women who were not deferred ($p < 0.0001$).[93]

Several large observational studies have evaluated risk factors for iron depletion in blood donors. The RISE study[66,89] enrolled and tracked individuals who had not previously donated or not within the prior two years (first-time and reactivated donors, FT/RA), and another cohort consisting of frequent repeat donors (females with >2 donations or males with >3 donations in prior year) longitudinally over a 15–24-month period. At enrollment, female donors of reproductive age were 3–7 times more likely to have AIS, or plasma

ferritin ≤12 ng/ml, than menopausal women or male donors. The prevalence of AIS was 0% in FT/RA male donors and 6.6% in FT/RA female donors; IDE was found in 2.5% males and 24% females. In the frequent donors, AIS was found in 16% males and 28% females; iron-deficient erythropoiesis (IDE) was found in 49% of male donors and 67% female donors. In statistical models controlling for demographic, behavioral, and other factors, donation intensity stood out as the most important predictor by far, with those donating 10 or more times over the prior two years 19 times more likely to have AIS than first-time donors and 50 times more likely to have IDE, an intermediate degree of iron depletion. The importance of donation frequency as a contributor to iron depletion in blood donors was highlighted at follow-up nearly two years later, when the prevalence of AIS in the FT/RA donor cohort had tripled in women (from 6.6 to 20%) and rose from 0 to 8% in men; higher increases were noted for IDE.[89] At the end of the study, the overall prevalence of AIS and IDE remained the same within frequent repeat donor cohorts. Donation intensity and female sex were found to be the strongest independent predictors of AIS and/or IDE. Age, weight, smoking, HFE genotype, menstrual, and pregnancy status were less strongly associated. Waiting longer intervals between donations (up to 14 weeks) was associated with lower risk for AIS compared to donations made at shorter intervals, as was taking self-directed iron supplements alone or in a multivitamin combination (reported in 39%). Diet had little or no impact.

A Danish study of nearly 15,000 blood donors also found high rates of iron depletion (ferritin < 15 ng/ml) in individuals donating three times per year for several years: 9% of men, 39% of premenopausal women, and 22% of postmenopausal women.[94] Iron deficiency was strongly associated with sex, menopausal status, blood donation frequency, and the time since the previous donation. The risk of iron depletion was only weakly associated with body weight, intensity of menstruation, and dietary and supplemental iron intake.

Genetic assessment of iron status in blood donors

A novel area of research that may help guide blood donor management in the future involves identifying common molecular polymorphisms that impact iron homeostasis.[52] In hereditary hemochromatosis (HH), defects in several iron pathway mediators or hepcidin leads to excessive accumulation of iron in the body. The FDA has approved a variance allowing blood centers to collect blood from individuals with HH for allogeneic transfusion, if certain requirements are met.[95] In addition, a transferrin polymorphism (G277S mutation) has been described that predisposes individuals to the development of iron deficiency.[96] Despite these considerations, no appreciable effect has been observed involving the transferrin G277S or heterozygous HFE mutations and iron status in blood donors.[97] A variant polymorphism of hypoxia-inducible factor-1α (HIF1α) has been reported to allow more donations without being deferred for low hemoglobin in male donors.[98] Novel polymorphisms involving TMPRSS6 are also being investigated.[99]

Adverse effects of iron depletion

It is well established that iron deficiency anemia results in fatigue and diminished exercise and work capacity. However, there is now increasing evidence that iron deficiency may have adverse effects even without anemia, related to the role of iron in metabolic pathways in the central nervous system and muscle tissue. Fatigue,[100] decreased exercise capacity,[101] pica,[102] restless legs syndrome (RLS),[103] and decreased cognitive performance[104,105] have been reported in association with non-anemic iron deficiency. A survey following blood donation found that fatigue was the third most common adverse event, resulting in a 20% reduction in donor return rates at one year.[106] Women who are fatigued but non-anemic have benefited from iron therapy.[100,107] In one study, premenopausal women with ferritin levels less than 50 ng/ml were randomized to receive intravenous iron or placebo.[100] Fatigue scores were significantly improved after therapy in the subgroup of women with ferritin under 15 ng/ml. In a study of oral iron therapy (80 mg/day elemental iron, ferrous sulfate), non-anemic women with unexplained fatigue in a general practice were found to have reduced fatigue scores after one month of treatment, but only in those with a baseline ferritin level <50 ng/ml.[107]

Fatigue has not been extensively studied in blood donors; however, in a study of 154 non-anemic female blood donors given 80 mg elemental iron versus placebo, fatigue scores were no different in the iron and no-iron groups when measured one month after blood donation and treatment.[108] However, it should be noted that this study was designed to evaluate the acute effect of low ferritin after blood donation. Pre-donation ferritin levels were 36.3 and 34.1 ng/ml in the iron and placebo groups, respectively (i.e., not in the iron-deficient range). Thus, the study did not evaluate the chronic effects of low iron and fatigue in blood donors.

Pica is an eating disorder characterized by the compulsive ingestion of nonfood substances—mostly ice (pagophagia), but including other substances such as chalk, clay, or uncooked starch—that also has been strongly associated with iron deficiency.[109] In the RISE study, pica symptoms were found in 6% of donors when surveyed at end of study, and in a multivariate analysis was eight times more common in women who had ferritin ≤12 ng/ml than in iron-replete women.[103] At the NIH, pica was found in 11% of iron-depleted or -deficient donors (ferritin < 20 ng/ml in women and <30 ng/ml in men) compared to 4% of iron-replete donors($p < 0.0001$). Pica symptoms resolved generally within two weeks of starting oral iron therapy.[102] Pica symptoms predominated in female donors in these studies.

RLS is a neuromuscular movement disorder in which patients complain of crawling, aching, or burning sensations in their legs, associated with a compelling urge to move their extremities to relieve the discomfort. Because the symptoms may appear or intensify at rest, the movement disorder may result in sleep disturbances. RLS has been associated with both iron deficiency anemia and non-anemic iron deficiency, which suggests a role for iron in dopaminergic pathways and metabolism.[110] A study in Sweden found the prevalence to be 15% in male donors and 25% in female donors, significantly higher than the prevalence of 6% in men and 11% in women reported in the general population.[111] Using questionnaire data, the RISE study found probable RLS in 9% of donors and possible RLS in 20%; however, there was no correlation with low ferritin levels.[103] At the National Institutes of Health (NIH), restless legs syndrome was reported in 16% of iron depleted or deficient donors and 11% of iron-replete donors ($p = 0.012$). Clinical improvement took longer than in donors with pica, requiring at least 4 to 6 weeks of therapy.[102]

Evidence of neurocognitive impairment resulting from iron deficiency and reversal with iron treatment suggests an important

role of iron in central nervous system function.[104,112] In addition, long-term studies of teenaged subjects have shown that iron depletion during adolescence is associated with micro- and macro-neuroanatomical changes measured by high-resolution magnetic resonance imaging (MRI) scanning in early adulthood,[113] reinforcing concern about possible effects of iron deficiency on fetal neurological development in female blood donors who become pregnant. Iron supplementation has also been shown to improve maximal and submaximal exercise performance in premenopausal women.[114] Taken together, these studies suggest that iron depletion may have deleterious consequences in blood donors and that measures to monitor and prevent iron depletion are warranted.

Iron present in excessive amounts in the body may be toxic due to the formation of iron-induced free radicals and lipid peroxidation.[115] Some studies of adults have reported that high iron stores are associated with increased risk for coronary heart disease,[116] implying that low iron levels may be salutary and that blood donation may be beneficial. However, this notion has not been substantiated in large population-based ferritin studies or epidemiological studies of blood donors.[117,118] The age-adjusted relative risk (RR) of myocardial infarction for men in the highest category of blood donations (>30 lifetime; mean ferritin from subset of subjects, 64 ng/ml) compared with never donors (mean ferritin, 187 ng/ml) was 1.2 (95% CI, 0.8 to 1.8), and this risk was not significantly changed after adjustment for coronary risk factors or subgroup analyses limited to men with hypercholesterolemia or those who never used antioxidant supplements or aspirin.[118] In a trial of serial phlebotomy to lower ferritin levels in men with peripheral arterial disease, there was no effect on all-cause mortality (HR, 0.85; 95% CI, 0.67–1.08) or the incidence of myocardial infarction and stroke (HR, 0.88; 95% CI, 0.72–1.07).[119] A comprehensive review of epidemiologic and intervention studies concluded that existing evidence does not indicate a link between ferritin and cardiovascular disease risk.[120]

Mitigation of iron deficiency

Increased interdonation interval

Observational studies suggest that prolonging the interdonation interval reduces the risk of iron depletion in blood donors. The RISE study found that interdonation intervals of less than 14 weeks were associated with increased risk of low hemoglobin deferral (OR ~ 2–2.5) and iron deficiency (OR ~ 3–4.4 for ferritin <12 μg/L), and concluded that lengthening the minimum interval would reduce the risk of iron depletion.[89] However, because 39% of donors in RISE acknowledged taking some form of iron supplements, iron ingestion (and not simply waiting longer between donations) may also have played a role in shortening the period of iron recovery. A Norwegian study in non-iron-supplemented donors followed for 1 year also described decreased ferritin levels in both sexes and decreased hemoglobin levels in females, suggesting in their model that increased donation intervals (from a three-month minimum) may protect against iron deficiency.[121] However, studies also show that prolonging the minimum donation interval (changing from 8 weeks to 12 or 16 weeks) might reduce the blood supply (especially for critically needed O-negative blood type) in the United States by as much as 5–7%.[122]

Although intended to protect blood donors from iron deficiency and anemia, there is wide variation in international standards that regulate the allowable frequency of blood donation involving red blood cells.[54] Previous studies that led to the establishment of an eight-week interdonation interval were based on research using small numbers of young, iron-replete subjects,[123] or on limited data on regular blood donors before the availability of reliable iron measurements.[124] A more contemporary study using a quantitative carboxyhemoglobin technique found that hemoglobin from 550 ml donated blood was replenished in 36 +/− 11 days after donation, but it also lacked generalizability because it was restricted to iron-replete young men.[125] Recovery of hemoglobin and iron after donating a unit of whole blood with and without iron supplementation was tested directly in the Hemoglobin and Iron Recovery Study (HEIRS) protocol.[126] The investigators enrolled over 200 blood donors (men and women, 18 to 79 years old) who had not donated for at least four months, measured their iron (ferritin) levels, and divided them into two groups: a low-ferritin group (iron-depleted, plasma ferritin ≤26 ng/ml) and higher ferritin group (non-iron-depleted, plasma ferritin >26 ng/ml). They randomized half of each group to take low-dose iron tablets, 37.5 mg daily, for 24 weeks, and monitored hemoglobin and ferritin levels at regular intervals to see how fast they recovered. Hemoglobin and ferritin recovery were delayed in both untreated ferritin groups (Figures 5.5 and 5.6): the low-ferritin group required a mean of 158 days and the higher ferritin group required a mean of 78 days to recover 80% of the hemoglobin they lost at the time of donation. Without iron, two-thirds of the donors did not recover the iron they lost by 24 weeks. Thus, although extension of the minimum interval would by definition allow for more complete recovery between donations, an increase in the interdonation interval to as long as 24 weeks would still not provide adequate time for hemoglobin and iron recovery in many donors.

Ferritin testing

Studies examining the impact of providing ferritin results to blood donors are limited. Investigators in Switzerland conducted a large single-center longitudinal study to evaluate routine ferritin testing in blood donor management.[127] Serum ferritin was assessed at each blood donation in over 160,000 donations (approximately 24,000 donors) from 1996 to 2009 starting in 2004. Ferritin below 10 ng/ml was the threshold considered to be iron depleted, leading to medical counseling by a blood bank physician to assess other potential medical reasons for low iron or to be referred to their physician, and to considering measures to improve iron status such as reducing donation frequency and/or taking supplemental iron. Comparing groups before and after institution of screening ferritin in donors revealed (1) increased hemoglobin levels, 0.26 g/dl in women and 0.19 g/dl in men; (2) reduced prevalence of anemia: using WHO criteria, hemoglobin <12 g/dl (women) or 13 g/dl (men), from 3.6% to 2.2% in women, and from 0.7% to 0.5% in men; and (3) using reduced hemoglobin deferrals (using hemoglobin <12.3 g/dl in women, and <13.3 g/dl in men), deferral rate went from 2.8% to 1.9% in women. The donor return rate declined as well, from 72–75% before to 60–64% after institution of ferritin screening. This study shows that ferritin monitoring can reduce anemia and hemoglobin deferral in blood donors; however, some donors stop donating when informed of their ferritin results. Of some interest, the investigators have discontinued routine ferritin screening, deciding on a more targeted strategy incorporating annual ferritin testing.[128]

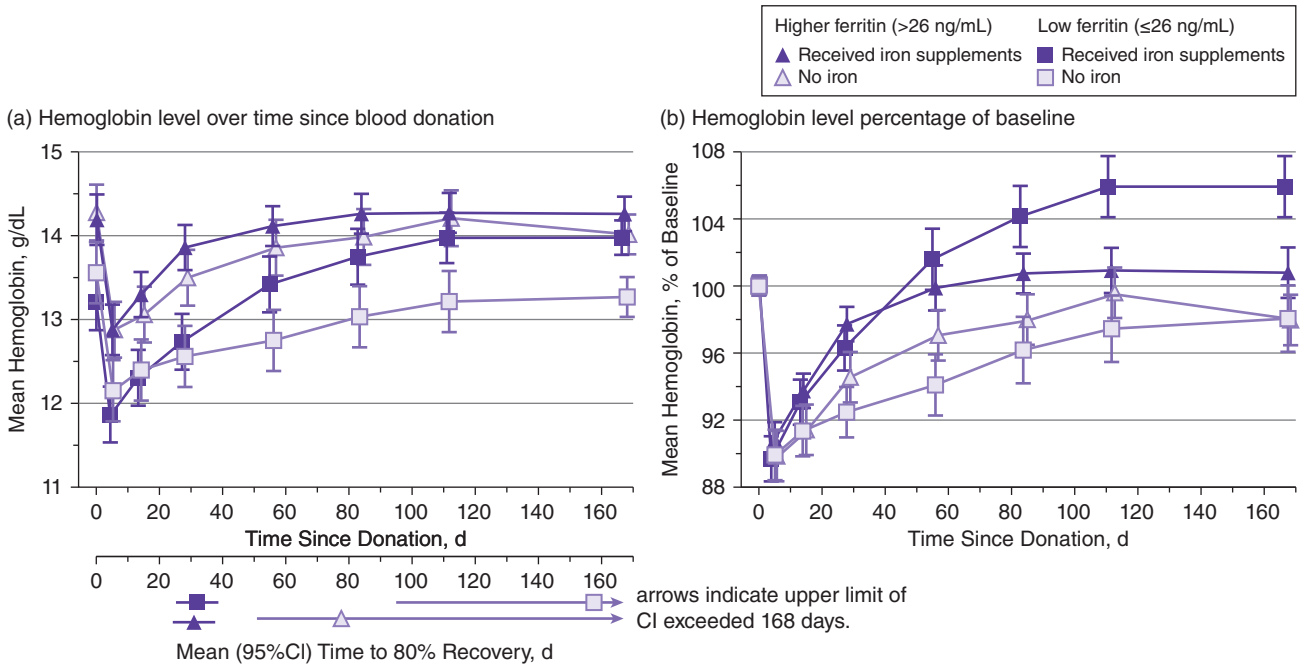

Figure 5.5 Hgb recovery after a blood donation and the effect of iron supplementation. Source: Kiss *et al.* (2015).[126] Reproduced with permission of American Medical Association.

In a multivariable analysis, the Danish Blood Donor Study Group found the strongest predictors of low hemoglobin concentration and risk of declining hemoglobin (at least 0.5 gm/dl) were low ferritin (<15 ng/mL) and current use of iron supplementation (an unexpected association with *lower* hemoglobin was considered to be because of routine iron prescription in the event of low hemoglobin deferral).[129] As the strongest predictor of hemoglobin level, "regular" ferritin measurement was felt to be the most informative tool for blood donor management; however, additional studies are needed to evaluate how often ferritin testing should be performed and what treatment should be offered. A pivotal study, Strategies to Reduce Iron Deficiency (STRIDE), is designed to determine the effectiveness of donor education (two arms: "Thank you" or ferritin result) and oral iron supplementation (three arms: placebo, 19 mg, or 38 mg iron tablets) for mitigating iron deficiency in frequent blood donors.[130] In the preliminary results, withdrawals within two months of enrollment occurred more frequently among donors receiving tablets than those receiving letters, with iron status outcomes pending publication of final results.

Iron supplementation

A number of trials have shown that iron deficiency can be prevented and/or ameliorated by providing iron supplements to blood donors.[131–135] Many studies have targeted premenopausal women because of the disproportionate prevalence of iron deficiency in this donor population. A study performed in Australia reported that, for female donors aged 18–45 who had mean baseline ferritin values ~31 ng/ml and who took 45 mg elemental carbonyl iron daily for eight weeks, ferritin levels at week 12 were significantly higher in donors receiving carbonyl iron (17.0 ± 10.9 ng/mL) compared with those receiving placebo (10.6 ± 8.4 ng/mL; $p < 0.001$), and the proportion of iron-deficient donors (ferritin ≤15 ng/ml) was significantly lower in the carbonyl iron group (51.9%) compared to the placebo (80.5%; $p < 0.001$).[133] Excluding darker stool color, 31.2% of the carbonyl iron group reported at least one GI side effect, compared with 25.5% of the placebo group. 85% of the participants responded that they would be willing to take iron supplementation in the future. An additional study of iron replacement in female blood donors <50 years old with iron deficiency without anemia showed that four weeks of oral ferrous sulfate, 80 mg of elemental iron/day, resulted in a significantly lower prevalence of a ferritin <12 ng/mL compared to donors given placebo.[134] A study in very frequent blood donors of both sexes (men up to six times and women up to four times per year) who were randomized to 40 mg, 20 mg, or placebo ferrous gluconate found that the ferritin levels remained low but were maintained in donors who received 20 mg daily but declined in the placebo controls.[135] Positive iron balance (increased ferritin) was observed only in donors taking 40 mg daily. In the HEIRS trial, compared to donors who did not take iron, donors taking iron supplements had a significantly shorter time to hemoglobin recovery in *both* the low-ferritin (mean 32 days vs. 158 days) and higher ferritin groups (31 days vs. 78 days).[126] The accelerated hemoglobin recovery from iron supplements was seen in donors with ferritin values up to 50 ng/ml, or higher than expected based on pre-donation ferritin. This is because of the iron deficit induced by blood donation, essentially lowering ferritin in some donors to iron-deficient levels post donation (ferritin <26 ng/ml; Figure 5.6), thus delaying their erythropoietic recovery. Above a ferritin of 50 ng/mL, donors no longer recovered hemoglobin any faster on iron supplements. With iron tablets, iron stores recovered in about 11 weeks, slightly longer than the current eight-week waiting period for donors to be eligible to donate again. Without iron, two-thirds of the donors did not recover the iron they lost by the end of the study in 24 weeks.

A meta-analysis of iron supplementation has reported results of 30 randomized controlled trials involving 4704 blood donors to

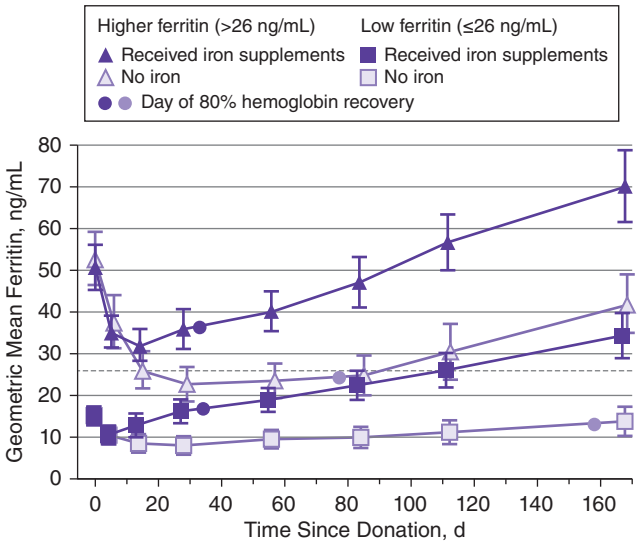

Figure 5.6 Ferritin recovery after blood donation. Source: Kiss *et al.* (2015).[126] Reproduced with permission of American Medical Association.

reduce low hemoglobin deferral, anemia, and iron deficiency.[136] The systematic review concluded there was moderate-quality evidence of "considerably lower" rates of deferral (RR, 0.34; 95% CI, 0.21, 0.55; $n = 1194$, $p < 0.0001$) and improved hemoglobin levels (mean increase, 0.24 gm/dl; 95% CI, 0.01, 0.47; $n = 847$, $p = 0.04$) and iron stores (mean ferritin increase, 14.0 ng/ml; 95% CI, 8.9, 19.0; $n = 640$, $p < 0.00001$) in supplemented donors at the first donation, which persisted at subsequent donations. Adverse effects (constipation, diarrhea, nausea, vomiting, and taste disturbance) were also higher (RR, 1.60; 95% CI, 1.23, 2.07; $n = 1748$, $p = 0.0005$), which prompted the authors to caution about potential limitations in widespread supplementation programs. However, the dosage, frequency (up to tid), and duration of therapy (up to one year) in some studies were high, and this may have increased the incidence of adverse events. The investigators concluded that targeted use of supplements (in those at highest risk) should be considered, with tailored donation schedules and dietary advice in others. The compelling reduction in hemoglobin deferral rate by approximately two-thirds should translate into improved donor retention and decreased donor recruitment resource expenditures at blood centers.

Studies targeting hemoglobin-deferred donors have also been reported. At the NIH, approximately 1200 blood donors deferred for a low hemoglobin and 400 nondeferred donors were evaluated for iron depletion (defined as a ferritin level of 9–19 mg/L in women and 18–29 mg/L in men) and deficiency (as defined by serum ferritin levels below the institutional reference range of 9 ng/ml in women and 18 ng/ml in men).[92] Iron depletion or deficiency was found in 53% of females and 61% of males who were deferred versus 39% of female and 39% of male nondeferred donors. Individuals who were iron deficient or deferred received 60 tablets of 325 mg oral ferrous sulfate (65 mg elemental iron) and instructed to take one daily (and again at subsequent donations). Iron-related laboratory measurements improved in all donors despite continued blood donations. An operationalized approach to implementing an iron replacement program to mitigate iron deficiency in deferred female donors has also been described.[137]

Although more blood centers have begun to advise frequent donors to take supplements to replace iron lost with the donation, iron supplementation may not be more widely recommended for several reasons. First, it may be that side effects (or simply taking a pill regularly) have a limiting effect on a donor's willingness to ingest oral iron. Conventional doses as used in clinical iron deficiency anemia have been poorly tolerated with up to 80% GI side effects and frequent donor dropout.[138] Several studies have suggested that a 2000 mg elemental iron total dose for short-term replacement after blood donation is effective[139–142] and that a 40 mg daily dose given as ferrous iron salt over an eight-week period can achieve this therapeutic outcome.[106] Doses of 18–45 mg elemental iron daily (regardless of congener, e.g., sulfate, gluconate, and carbonyl) are generally tolerated with occasional GI symptoms such as nausea, bloating, cramps, and constipation, and with advance notice about having darker stools.[126,133,135] In the NIH study, initial use of 65 mg elemental iron as ferrous sulfate resulted in unacceptable side effects in 21% of donors, with successful resolution in some individuals after switching to 38 mg elemental iron tablets (ferrous gluconate).[92] Concern by blood center personnel also remains in regard to recommending or providing iron to individuals with undiagnosed hemochromatosis and potentially masking anemia due to occult blood loss from the GI tract, possibly delaying the discovery of a GI illness.[143] Steps to address these important issues, especially donors with a family history of iron overload, a personal or family history of colon polyps or cancer, and severe anemia or failure to improve hemoglobin after taking oral iron, should be considered as part of donor education and informed consent.

In summary, iron deficiency is widespread in blood donors, and certain groups of donors are particularly likely to become iron depleted, including premenopausal women (with ongoing menstrual blood losses), and "frequent" blood donors including women who donate two or more times a year, and men who donate three or more times a year. Although questions remain regarding how best to manage this problem, solutions are beginning to emerge. It is evident that simply waiting longer between donations (i.e., increase the interdonation interval) is not particularly effective, because the amount of iron removed in the 10 minutes or so it takes to donate a unit of blood requires over 24 weeks to replace on a "standard" diet (i.e., without added iron in the form of supplements). The cumulative effect of repeat blood donations without adequate iron replacement results in iron deficiency in many donors and anemia in some. On the other hand, low-dose iron supplements (18–45 mg elemental iron, available over the counter) accelerate hemoglobin and iron recovery, reduce hemoglobin deferrals, and appear to be tolerated fairly well by most blood donors. Ferritin screening has also been shown to improve hemoglobin levels and iron status; however, iron supplementation has also been recommended in conjunction with this approach and is likely to account for the salutary benefits reported in these studies.

Conclusion

Whole blood donation and apheresis procedures to collect blood components have an impressive safety record, and most volunteer blood donors have uneventful donations and feel good about donating blood to help others. Still, blood donation is a procedure associated with risk of minor discomfort and pain, iron depletion, and, in rare cases, serious injury. Recent operational trials and research programs identify possible ways to reduce the risk of complications and iron depletion after blood donation. Ongoing efforts and continued vigilance are necessary to further improve safety for volunteer blood donors.

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A full reference list for this chapter is available at: http://www.wiley.com/go/simon/transfusion

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