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Iron Stores and Vascular Function in Voluntary Blood Donors

Haoyi Zheng, Ritchard Cable, Bryan Spencer, Nancy Votto, Stuart D. Katz

Background—Iron is a pro-oxidant cofactor that may be linked to atherosclerosis progression. Reduction of body iron stores secondary to blood donation has been hypothesized to reduce coronary risk, but retrospective studies have yielded inconsistent findings. We sought to assess the effects of blood donation frequency on body iron stores and physiological and biochemical biomarkers of vascular function associated with atherosclerosis progression.

Methods and Results—Forty high-frequency voluntary blood donors (≥ 8 donations in past 2 years) and 42 low-frequency blood donors (1 to 2 donations in past 2 years) aged 50 to 75 years were randomly selected from American Red Cross of Connecticut blood donor records. Flow-mediated dilation in the brachial artery, serum markers of iron stores, vascular inflammation and oxidative stress, and cardiac risk factors were assessed in all subjects. Serum ferritin was significantly decreased in high-frequency blood donors when compared with low-frequency blood donors (median values 17 versus 52 ng/mL; $P < 0.001$), but hematocrit did not differ between groups. Flow-mediated dilation in the brachial artery was significantly greater in high-frequency donors when compared with low-frequency donors in univariate analysis ($5.5 \pm 2.6\%$ versus $3.8 \pm 1.6\%$; $P = 0.0003$) and in multivariate analysis adjusting for cardiac risk factors and other potential confounders. Serum biomarkers of vascular inflammation did not differ between groups but 3-nitrotyrosine, a marker of oxidative stress, was decreased in high-frequency donors when compared with low-frequency donors.

Conclusions—High-frequency blood donors had evidence of decreased body iron stores, decreased oxidative stress, and enhanced vascular function when compared with low-frequency donors. These findings support a potential link between blood donation and reduced cardiovascular risk that warrants further investigation in prospective outcome studies. (*Arterioscler Thromb Vasc Biol.* 2005;25:1577-1583.)

Key Words: ferritin ■ nitric oxide ■ oxidative stress ■ vascular endothelium ■ vasodilation

Iron is a pro-oxidant cofactor associated with increased production of hydroxyl radical in cardiovascular tissues and increased progression of atherosclerosis in experimental models.¹⁻⁴ Reduction of body iron stores secondary to blood donation has been hypothesized to reduce coronary heart disease risk.^{5,6} However, previous epidemiological studies on the association of blood donation and cardiovascular risk have yielded inconsistent findings.⁷

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The vascular endothelium releases numerous bioactive substances that regulate vasomotor tone, vascular structure, blood vessel-platelet interactions, and fibrinolysis/thrombolysis balance.⁸ Dysfunction of the vascular endothelium in response to inflammation and oxidative stress is thought to be an important determinant of atherosclerosis progression.⁹ Physiological and biochemical markers of vascular endothelial dysfunction are associated with increased risk of cardiac events.^{10,11} Previous clinical studies have demonstrated that acute reduction of iron bioavailability with chelating agents

improves vascular endothelial function, but the effects of chronic reduction in iron stores caused by blood donation on vascular function have not been previously described.^{12,13} We hypothesized that high-frequency blood donation would be associated with greater reduction in body iron stores and improved vascular function when compared with less frequent donation. Accordingly, we sought to determine the effects of high-frequency versus low-frequency blood donation on serum markers of body iron stores, serum markers of vascular inflammation and oxidative stress, and flow-mediated dilation in the brachial artery of voluntary blood donors.

Methods

Study Sample

Currently eligible voluntary blood donors were recruited for the study based on blood donation records from the American Red Cross. High-frequency blood donors (at least 8 donations in past 2 years) and low-frequency blood donors (at least 2 donations over the past 10 years with not >1 donation/year for the past 2 years) were eligible for the study. Subjects with a history of major bleeding

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events (including trauma, surgery, and menstruation or other uterine bleeding) within the past 2 years, diabetes mellitus, previous myocardial infarction, cancer or active chronic inflammatory disease, or tobacco use within 6 months were excluded. Subjects aged 50 to 75 years residing in New Haven County, Connecticut with blood donation history consistent with study entry criteria were identified from American Red Cross records and were randomly selected for invitation to participate in the study by written communication. All subjects provided written informed consent before participation in the study. The study protocol was approved by Human Investigation Committee of Yale University and the American Red Cross Institutional Review Board.

Study Protocol

Studies were performed in the morning after a 12-hour fast at least 4 weeks after the most recent blood donation. Subjects were instructed to avoid caffeine for 24 hours before the study visit. Background medications were delayed on the morning of the study until after completion of study procedures. After participants provided written informed consent and written release for their American Red Cross blood donation history, information on cardiovascular risk factors, prescription medication and supplement use, dietary heme-iron ingestion (frequency of ingestion of red meats, shellfish, and sardines), occupational history, and physical activity level were obtained, and heart rate and blood pressure were recorded in the supine position.^{14,15} Flow-mediated vasodilation was measured in the brachial artery with noninvasive ultrasound imaging as described. After completion of the imaging study, 20 mL of blood was obtained from a forearm vein for laboratory studies as described.

Endothelial Function Testing

Flow-mediated dilation in conduit arteries is partly attributable to endothelium-derived release of the potent vasodilator substance nitric oxide in response increased arterial wall shear stress.¹⁶ Flow-mediated dilation in response to posts ischemic hyperemia was determined noninvasively with high-resolution ultrasound imaging of the brachial artery with a 11.5-MHz linear array transducer connected to a duplex ultrasound system (SonoSite, Inc, Bothell, Wash) adapted from previously published guidelines.^{13,16} Ultrasound data acquisition and analysis were performed without knowledge of blood donation status. Arterial diameter (cm) was determined as the internal dimension of the vessel wall, from trailing edge to leading edge of the anterior and posterior intimal markings, respectively. Brachial artery blood flow velocity was determined with a 1.2-mm pulsed Doppler ultrasound sampling volume placed in the center of the image of the vessel lumen with internal software correction for the incident angle of 60°. Mean blood flow velocity (cm/sec) was determined by calculation of the area under the hand-traced curve of the velocity spectral display for each cardiac cycle. Brachial artery diameter and blood flow velocity were measured at rest and after release of transient arterial occlusion induced by inflation of a forearm pneumatic blood pressure cuff to suprasystolic pressure for 5 minutes. Blood flow velocity was measured for 15 seconds immediately on release of the occluding cuff; the mean velocities of the first 5 beats after release were averaged for calculation of the peak blood flow velocity. Brachial artery diameter was measured at end-diastole 60 to 75 seconds after release of the occluding cuff; 5 diameter measurements from a single cardiac cycle were averaged. Flow-mediated dilation was calculated as the percent change in brachial artery diameter after cuff release compared with the resting brachial artery diameter. Brachial artery flow was calculated by using the formula: flow (mL/min)=time-averaged mean velocity (cm/sec)×cross-sectional area (cm²)×60 sec/min.

Laboratory Studies

Complete blood cell count (Abbott Celldyne 3500 cell counter), serum iron and total iron binding capacity (Roche Diagnostics Modular P system), percent iron saturation (calculated from the ratio of serum iron to total iron binding capacity), and serum ferritin (immunoassay, DPC IMMULITE Chemiluminescent Analyzer)

were measured in the clinical laboratory at Yale-New Haven Hospital. Plasma glucose levels (Yellow Springs Instruments YSI 2700 STAT Analyzer), insulin levels (radioimmunoassay, Linco Laboratories), and lipid levels (AutoAnalyzer, model 747 to 200; Roche-Hitachi) were measured in the Yale General Clinical Research Center Laboratory. Insulin resistance was assessed with the homeostatic model assessment (HOMA-IR) as previously described: HOMA-IR=[fasting insulin concentration (U/mL)*fasting glucose concentration (mmol/L)/22.5].¹⁷ Plasma C-reactive protein was measured with high-sensitivity C-reactive protein reagent (Alfa Wassermann ACE; Clinical System). Total plasma homocysteine level was measured with Carolina Liquid Chemistries HCY reagent (Alfa Wassermann ACE, Clinical System). IL-6, soluble intercellular adhesion molecule-1, and 3-nitrotyrosine were measured with commercially available enzyme-linked immunosorbent assays (R&D systems, Oxis Research).

Data Analysis

Descriptive statistics were used to determine means and distributions of clinical characteristics and physiological and laboratory measurements in the study sample. Continuous variables with normal distributions are presented as mean±SEM in text and tables. Serum ferritin, high-sensitivity C-reactive protein levels, IL-6 levels, 3-nitrotyrosine levels, and body mass index were noted to significantly deviate from the normal distribution (by Shapiro-Wilk test) so the natural logarithm of these variables was used in all analyses; median values and interquartile range for these variables are presented in text and tables. Unpaired Student *t* tests and χ^2 tests were used to compare means and proportions of baseline clinical and laboratory characteristics respectively in high- and low-frequency blood donors. Univariate linear regression was used to determine the association between flow-mediated dilation, blood donation frequency, and clinical and laboratory variables. Multivariate linear regression models were used to determine the relationship between blood donation frequency and flow-mediated dilation with adjustment for potential confounding variables (Stata statistical software version 8.0; College Park, Tex). Because our sample size limited the number of variables in multivariate models, we developed 2 discrete models: model 1 was based on inclusion of traditional and nontraditional risk factors for coronary heart disease that are known to be associated with endothelial function (age, gender, history of hypertension or hyperlipidemia, systolic blood pressure, body mass index, serum low-density lipoprotein cholesterol level, plasma high-sensitivity C-reactive protein level, plasma homocysteine level), and model 2 was based on inclusion of clinical and laboratory variables from our data set that were associated with flow-mediated dilation with univariate $P<0.20$ (body mass index, homocysteine level, triglyceride level, HOMA-IR, log ferritin level, hematocrit, brachial artery rest diameter, and use of hydroxymethylglutaryl (3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors, diuretics, or β -blockers). For both models, we used forward stepwise regression with adjusted $P<0.20$ as the criterion for retention in the model. Plots of jack-knife residuals versus predicted values were used to check final model assumptions. For all analyses, a 2-tailed $P<0.05$ was used to infer statistical significance. From previous data derived from our laboratory, the mean and standard deviation of the flow mediated dilation measurement in healthy younger subjects was 5.89±2.88%. Based on this estimate of variance and 2-tailed $\alpha=0.05$, 40 subjects in each group (high-frequency versus low-frequency donors) provided >80% power to detect a clinically relevant 33% difference between blood donor groups.

Results

Clinical Characteristics

The total number of blood donations over 2 years and 10 years was significantly greater in the 40 high-frequency donors when compared with 42 low-frequency donors (2-year donation history 9.6±0.2 versus 1.6±0.1 U and 10-year donation history 35±2 versus 5±0.4 U, both $P<0.001$). The

TABLE 1. Clinical Characteristics of Study Sample (Percent, Mean±SEM, or Median Value With Interquartile Range) by Blood Donation Frequency

	High-Frequency Donors (n=40)	Low-Frequency Donors (n=42)	P*
Age, y	61±1	58±1	0.13
Sex, % male	73%	48%	0.02
Comorbidities, %			
Hypertension	50	37	0.22
Hyperlipidemia	58	27	0.005
Medications, %			
ACE inhibitor or ARB	28	15	0.16
β-blocker	10	15	0.53
Thiazide diuretic	15	10	0.47
HMG-CoA reductase inhibitor	45	22	0.03
Vitamin C	18	29	0.21
Multivitamin	68	63	0.70
Body mass index, kg/m ²	27.8 (6)	27.3 (6)	0.49
Systolic blood pressure, mm Hg	125±3	123±2	0.56
Diastolic blood pressure, mm Hg	75±1	75±1	0.98
Heart rate, min ⁻¹	65±1	66±1	0.47
Total cholesterol, mg/dL	180±5	188±5	0.24
LDL cholesterol, mg/dL	102±4	107±4	0.46
HDL cholesterol, mg/dL	59±3	62±2	0.41
Triglycerides, mg/dL	95±10	98±9	0.82
Glucose, mg/dL	97±1	97±1	0.95
Insulin, μU/mL	13.5±0.9	14.5±1.1	0.46
HOMA-IR	3.3±0.2	3.5±0.3	0.49
Homocysteine, ng/mL	10.5±0.4	10.6±0.5	0.86
hs-CRP, mg/dL	0.9 (1.2)	1.2 (3.0)	0.23

ACE indicates angiotensin-converting enzyme; ARB, angiotensin receptor blocker; HDL, high-density lipoprotein; HMG, hydroxymethylglutaryl; HOMA-IR, homeostatic model of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein.

*P for unpaired *t* test or χ^2 test of high-frequency vs low-frequency donor groups.

clinical characteristics of the high-frequency blood donors and low-frequency blood donors are summarized in Table 1. The high-frequency donor group had a greater proportion of males, a greater proportion of subjects with history of hyperlipidemia, and consequent greater use of 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors when compared with the low-frequency donor group. Dietary intake of heme-iron, use of iron supplements, physical activity scale, occupational history, highest level of completed education and family history of coronary artery disease did not differ in high-frequency versus low-frequency blood donors (data not shown, all probability values not significant).

Laboratory Markers of Iron Stores

Serum markers of iron stores were significantly decreased in high-frequency donors when compared with low-frequency donors (Table 2). Seventeen of the 40 high-frequency blood donors had evidence of severely reduced iron stores (serum ferritin <12 ng/mL) compared with 2 of the 42 low-frequency blood donors (44% versus 5%; $P<0.001$). The total number of blood donations over 2 years and 10 years

was significantly associated with lower serum ferritin levels ($r=-0.60$ and $r=-0.48$, respectively, both $P>0.001$). Despite increased prevalence of severely reduced iron stores in the high-frequency donors, hemoglobin and hematocrit did not differ in high-frequency versus low-frequency donors (Table 2). Mean corpuscular volume was significantly lower and red cell distribution width was significantly greater in high-frequency blood donors when compared with low-frequency blood donors (Table 2). Mean corpuscular hemoglobin concentration and white blood cell count did not differ between groups (Table 2). Platelet count tended to be increased in low-frequency donors when compared with high-frequency donors with borderline statistical significance (Table 2).

Vascular Function

Brachial artery diameter at rest and resting and postischemic hyperemic brachial artery blood flows did not differ in high-frequency versus low-frequency blood donors (diameter 3.77 ± 0.01 versus 3.65 ± 0.01 mm, $P=0.4$; rest flow 76 ± 7 versus 65 ± 5 mL/min, $P=0.24$; hyperemic flow 535 ± 37

TABLE 2. Blood Markers of Iron Status (Means±SEM or Median Value With Interquartile Range for Serum Ferritin) by Blood Donation Frequency

	High-Frequency Donors (n=40)	Low-Frequency Donors (n=42)	P
Serum ferritin, ng/mL	17 (15)	52 (48)	<0.001
Serum iron, mg/dL	75±6	84±3	0.19
Total iron-binding capacity, µg/dL	363±10	325±7	0.003
Iron-binding saturation, %	22±2	28±2	0.03
Hemoglobin, g/dL	13.9±0.2	14.1±0.2	0.35
Hematocrit, %	41±0.6	42±0.5	0.39
MCV, fL	88.0±0.9	90.3±0.5	0.03
MCHC, %	33.9±0.2	33.9±0.2	0.84
RDW	12.5±0.2	12.0±0.1	0.007
WBC count, ×1000 cells/mm ³	5.2±0.2	5.6±0.2	0.17
Platelet count, ×1000 cells/mm ³	253±8	278±11	0.06

MCHC indicates mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RDW, red cell distribution width; WBC, white blood cell.

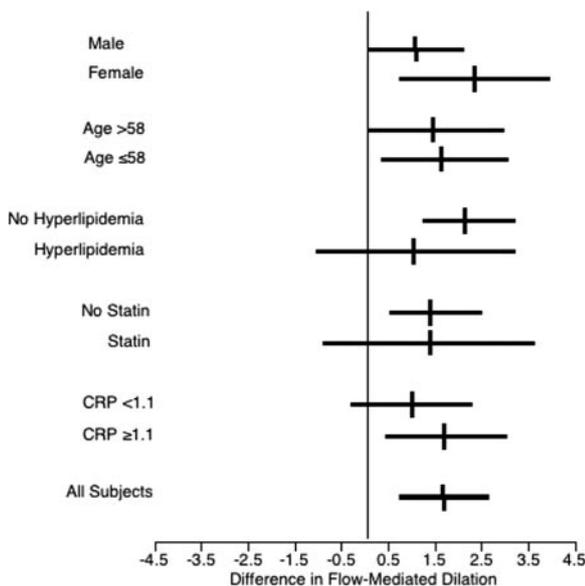
versus 605±48 mL/min, *P*=0.26). Flow-mediated dilation was significantly increased in high-frequency blood donors when compared with that of low-frequency blood donors (5.5±2.6% versus 3.8±1.6%, *P*=0.0003; Figure) and was significantly associated with the increased number of blood donations over 2-year and 10-year periods (*r*=0.39 and *r*=0.34, respectively, both *P*<0.01). Estimates of differences in flow-mediated dilation between high-frequency and low-frequency donors were consistent across subgroups (Figure). In multivariate models, high-frequency blood donation remained significantly associated with increased flow-mediated dilation when adjusting for established traditional and non-traditional cardiovascular risk factors (model 1, estimated

difference in flow-mediated dilation between high-frequency and low-frequency donor groups 1.49%; 95% confidence intervals 0.56% to 2.43%; *P*=0.002) and when adjusting for variables associated with flow-mediated dilation in univariate analysis with *P*<0.20 (model 2, estimated difference in flow-mediated dilation between high-frequency and low-frequency donor groups 1.68%; 95% confidence intervals, 0.71% to 2.65%; *P*=0.001). In a post-hoc analysis that excluded subjects with hypertension, hyperlipidemia or any prescription medication use, flow-mediated dilation in 10 high-frequency donors remained significantly greater than that of 21 low-frequency donors (6.1±0.5 versus 3.5±0.3%; *P*<0.001). Serum markers of vascular inflammation did not differ between high-frequency and low-frequency blood donors (IL-6 1.39 [0.96] versus 1.44 [1.07] pg/mL, soluble intercellular adhesion molecule-1 13.0±0.3 versus 12.2±0.4 ng/mL; probability values not significant). Serum levels of 3-nitrotyrosine, a marker of oxidative stress, were significantly decreased in high-frequency donors when compared with low-frequency donors (35 [21] versus 43 [37] nmol/L; *P*=0.02).

Discussion

The current findings demonstrate evidence of greater reduction of body iron stores, increased flow-mediated vasodilation in the brachial artery, and decreased oxidative stress in high-frequency blood donors when compared with that in low-frequency blood donors.

Sullivan first proposed in 1981 that chronic iron depletion from menstrual bleeding rather than gonadal hormone milieu may account for the reduced risk of coronary heart disease in premenopausal women.⁵ His original hypothesis was based on circumstantial evidence from epidemiological observations that demonstrated temporally coincident age-dependent increases in serum ferritin levels and increased coronary risk in both men and women with heterozygous familial hypercholesterolemia and from the observation that hysterectomy without oophorectomy was associated with increased coronary heart disease risk in women.¹⁸⁻²⁰ More recently, nega-



Estimates of differences in flow-mediated dilation (absolute difference in units of % with 95% confidence intervals shown) between high-frequency blood donors and low-frequency blood donors for all subjects and in subgroups defined by median age in years, gender, presence of hyperlipidemia, use of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) and median C-reactive protein (CRP) levels in mg/dL.

tive findings of controlled clinical trials of hormone replacement therapy in postmenopausal women indirectly support this alternative iron hypothesis.^{18,21,22} Experimental and clinical data provide a biologically plausible explanation for a link between iron metabolism and coronary heart disease risk. Iron is recognized to be a powerful pro-oxidant that is a necessary cofactor in the generation of hydroxyl radical in cardiovascular and other tissues.^{23–25} Laboratory investigations have demonstrated iron-dependent generation of reactive oxygen species in endothelial cell culture and increased aortic atherosclerosis in the apolipoprotein E-deficient mice and cholesterol-fed rabbits with increased iron intake.^{1–4} In clinical studies, chelation of iron with deferoxamine and dexrazoxane acutely improved endothelial function in subjects with coronary artery disease and in normal subjects with homocysteine-induced endothelial dysfunction.^{12,13}

The therapeutic corollary to Sullivan's initial hypothesis is that serial blood donation to mimic the severe reduction in iron stores found in menstruating females will reduce coronary heart disease risk. Blood donation is known to reduce total body iron stores as measured by serum ferritin levels. In healthy men, a single 500-mL whole blood donation results in a substantial loss of heme iron (≈ 200 to 250 mg) and 1 whole blood donation per year decreases serum ferritin levels by 44%.^{26,27} Epidemiological studies investigating the relationship between blood donation and coronary heart disease risk have yielded inconsistent findings.^{7,28–31} Misclassification of blood donation status because of recall bias, incomplete characterization of the study subjects with regard to body iron stores and cardiovascular risk factors, failure to exclude comorbid conditions that alter serum ferritin levels, and the presence of unidentified confounders related to the use of nonblood donor control groups limit interpretation of these previous studies.⁶ Moreover, few high-frequency blood donors with severe reductions in iron stores were included in past studies. The current study was designed to specifically address these limitations of prior investigations. Blood donation history in our subjects was objectively verified from American Red Cross records to eliminate recall bias. We selected active voluntary blood donors with less frequent donation as the comparator group to control for the potential confounding effects of the health status screening used by the American Red Cross to identify eligible blood donors. Many of our high-frequency donors had serum ferritin levels consistent with severe reduction in iron stores with a group mean serum ferritin value comparable to that observed in menstruating females.³² Our study sample was also extensively characterized with regard to clinical and laboratory variables known to be associated with coronary heart disease risk and flow-mediated dilation. Our findings are robust as high-frequency blood donation remained significantly associated with improved flow-mediated dilation when adjusting for multiple potential confounders in multivariate regression analysis and in a post-hoc analysis of the subgroup of subjects without cardiovascular risk factors or prescription medication use. Among the subjects without modifiable cardiovascular risk factors, flow-mediated dilation in low-frequency blood donors was below normal values for our laboratory, a finding likely attributable to increased age in this subgroup. In

contrast, flow-mediated dilation in high-frequency blood donors without modifiable cardiac risk factors was in the normal range for our laboratory. This finding suggests that high-frequency blood donation may protect against age-dependent attenuation in vascular function.

The mechanisms linking frequent blood donation to improved vascular function cannot be directly ascertained in the current cross-sectional study design. Our finding of decreased 3-nitrotyrosine in high-frequency donors is consistent with the hypothesis that reduction in iron stores is associated with decreased oxidative stress. 3-nitrotyrosine is derived from interactions between nitric oxide and superoxide anion in vascular tissues and other free radical chemistry reactions and is increased in experimental myocardial infarction and in patients with coronary artery disease.^{33–35} Whether increased flow-mediated dilation in our high-frequency donors is attributable to increased bioavailability of nitric oxide consequent to reduced oxidative stress and/or increased vascular smooth muscle responsiveness cannot be determined, because we did not measure nitroglycerin-mediated brachial artery dilation in our study. Although 3-nitrotyrosine levels as a biomarker of vascular oxidative stress were decreased in association with high-frequency blood donation, serum biomarkers of vascular inflammation did not differ between groups. This finding may be related to recognized limitations of the use of serum measurements as indicators of vascular wall cellular events but also raises the possibility that reduced iron stores may increase nitric oxide bioavailability without altering the inflammatory processes that contribute to atherosclerosis progression.⁹ Further work is needed to determine whether long-term reduction in iron stores is associated with reduced atherosclerosis progression.

Mechanisms other than reduction of iron-mediated oxidative stress may also contribute to our findings, because serum ferritin was not associated with flow-mediated dilation when adjusting for blood donation frequency in multivariate analysis. Although this finding could be attributed to the known limitations of serum ferritin as a marker of tissue iron stores,^{6,7,36} other plausible explanations of our findings exist. Hemoglobin levels did not differ according to blood donation frequency in our subjects, a finding consistent with American Red Cross procedures that exclude anemic patients from donation. However, our studies were performed at least 4 weeks after the most recent blood donation and small but statistically significant reductions in mean corpuscular volume were present in high-frequency donors. Transient small decreases in hematocrit after blood donation, chronic reductions from predonation baseline hematocrit, and/or small changes in red cell size may alter local arterial wall shear stress and/or nitric oxide–hemoglobin interactions and thereby modulate endothelial function.^{37–39} Changes in glucose metabolism related to iron stores may alter levels of glycohemoglobin that could also impact hemoglobin–nitric oxide interactions.^{40,41} Increased plasma erythropoietin levels in response to blood donation could also potentially alter endothelium-dependent vasodilation.^{42,43} Finally, it is possible that some unidentified confounder associated with the altruistic behavior of frequent voluntary blood donation may account for our findings. We attempted to minimize this

possibility by selecting active low-frequency blood donors as a control group and by extensively characterizing our study sample. We could not identify lifestyle differences related to the pattern of blood donation as body mass index, activity level, occupational history, level of education, dietary heme-iron intake, and use of iron and vitamin supplements did not differ in the high- versus low-frequency donors.

Our findings may have important implications for the health of blood donors and the development of strategies to increase recruitment of voluntary blood donors. Flow-mediated dilation in the brachial artery is a noninvasive biomarker of vascular function that has been previously reported to be related to endothelium-dependent vasomotion in the coronary circulation and to be significantly associated with clinical outcomes in cardiovascular disease populations.^{10,44–46} Coronary risk factors were common in our study sample with a median Framingham estimated 10-year cardiac risk of 5% (range <1% to 23%). Our finding of enhanced vascular function in association with frequent blood donation, even when adjusting for traditional and nontraditional coronary risk factors, suggests that regardless of underlying mechanism, high-frequency blood donation may be associated with reduced coronary risk. Additional prospective clinical outcome studies in larger populations are needed to test this hypothesis. A randomized, single-blind study of the effects of controlled phlebotomy on clinical outcomes in patients with peripheral vascular disease has completed recruitment of 1277 subjects with scheduled end of follow-up in April 2005.^{47,48} The current findings may assist in the interpretation of the results of this trial. Given the public health concerns related to chronic blood supply shortages in the United States and the strong safety record associated with blood donation, the current data could encourage voluntary blood donation and mitigate blood shortages pending availability of additional outcome data.

In conclusion, high-frequency blood donation was associated with evidence of reduction of body iron stores, improved vascular function, and reduced oxidative stress in voluntary blood donors. These findings lend support to the hypothesis linking body iron stores to cardiovascular disease risk. Additional prospective studies assessing cardiovascular outcomes in high-frequency blood donors are warranted.

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