Insulin Sensitivity, Vascular Function, and Iron Stores in Voluntary Blood Donors

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OBJECTIVE — Reduced iron stores after blood donation are associated with improved vascular function and decreased cardiovascular risk. We sought to determine whether iron-dependent changes in glucose metabolism may contribute to improved vascular function in blood donors.

RESEARCH DESIGN AND METHODS — We conducted a prospective cross-sectional study in 21 high-frequency blood donors (more than eight donations in the last 2 years) and 21 low-frequency blood donors (one to two donations in the last 2 years) aged 50–75 years. Serum markers of iron stores, whole-body insulin sensitivity index (WBISI) during oral glucose tolerance testing, and flow-mediated dilation in the brachial artery were determined in all subjects.

RESULTS — Serum ferritin was decreased (median values 23 vs. 36 ng/ml, $P < 0.05$) and flow-mediated dilation in the brachial artery was increased (median values 5.9 vs. 5.3%, $P < 0.05$) in high-frequency donors compared with low-frequency donors, respectively, but WBISI (median values 4.8 vs. 4.7) and related measures of glucose tolerance did not differ between groups. Flow-mediated dilation significantly decreased at 1 h after oral glucose loading in both groups, but the decrease in flow-mediated dilation at 1 h did not differ between high- and low-frequency donors.

CONCLUSIONS — High-frequency blood donation reduced serum ferritin and increased flow-mediated dilation compared with low-frequency donation but did not improve insulin sensitivity or protect the vascular endothelium from the adverse effects of acute hyperglycemia after oral glucose loading. These findings suggest that the mechanisms linking blood donation to improved vascular function are not likely related to changes in glucose metabolism.

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Iron is a prooxidant cofactor that has been proposed to be associated with increased risk of cardiovascular events in humans (1,2). Chronic reduction in iron stores in response to frequent blood donation is associated with improved vascular function in the brachial artery and biochemical evidence of decreased oxidative stress (3). In a randomized trial of patients with peripheral vascular disease, reduction of iron stores with serial phlebotomy decreased mortality compared with conventional therapy in the subjects in the youngest age quartile (aged 43–61 years) (4).

A potential mechanistic link between iron stores, vascular function, and cardiovascular risk may be related to iron-dependent effects on glucose metabolism (5,6). Diabetes is a well-recognized modifiable cardiovascular risk factor associated with increased serum ferritin levels and evidence of vascular endothelial dysfunction (7,8). Iron overload is associated with decreased insulin production, increased insulin resistance, and increased risk of diabetes (5,6). We hypothesized that improved vascular function associated with reduction in iron stores after blood donation might be partly attributable to improved insulin sensitivity. To test this hypothesis, we assessed insulin sensitivity during an oral glucose tolerance test and measured flow-mediated dilation in the brachial artery in a prospective cross-sectional study of nondiabetic high- and low-frequency voluntary blood donors.

RESEARCH DESIGN AND METHODS

— High-frequency (more than eight donations in the last 2 years) and low-frequency (one to two donations in the last 2 years) blood donors aged 50–75 years were identified from American Red Cross records and randomly selected for written invitation to participate in the study. Subjects with a history of major bleeding events within the last 2 years, BMI >40 kg/m^2, known diabetes, previous myocardial infarction, cancer or active chronic inflammatory disease, tobacco use within 6 months, or current use of medications known to influence glucose tolerance were excluded. The study protocol was approved by the human investigation committee of Yale University and the American Red Cross institutional review board. All subjects provided written informed consent before participation in the study.

Height, weight, hip/waist circumference, resting supine blood pressure and heart rate, and brachial artery flow-mediated dilation were measured. After completion of the baseline measurements, oral glucose tolerance testing was performed as described below. Brachial artery flow-mediated dilation was measured at 1 and 3 h during the oral glucose tolerance test. Body composition measured with dual-energy X-ray absorptiometry was completed on a second study day within 1 week of the oral glucose tolerance testing.

Oral glucose tolerance testing

After an overnight fast, 75 g oral glucose was administered (Orangedex; Custom Laboratories, Baltimore, MD). Blood was drawn from an indwelling catheter in a forearm vein before and at 30, 60, 90,

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Abbreviations: HOMA-IR, homeostasis model assessment of insulin resistance; WBISI, whole-body insulin sensitivity index.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Study sample characteristics by blood donation frequency*

	Low-frequency donors	High-frequency donors
n	21	21
Donations in last 2 years	2 (1)	9 (3)*
Age (years)	56 (7)	57 (4)
Sex (male/female)	13/8	12/9
Race (white/black/Asian)	19/1/1	21/0/0
BMI (kg/m^2)	26.5 (5.1)	26.5 (4.6)
Overweight or obese (*n*)	14	14
Waist circumference (cm)	93 (12)	92 (16)
Hip circumference (cm)	105 (14)	104 (11)
Percent body fat	29 (8)	31 (10)
LDL cholesterol (mg/dl)	102 (38)	97 (33)
HDL cholesterol (mg/dl)	57 (13)	59 (18)
Triglycerides (mg/dl)	68 (54)	64 (53)
C-reactive protein (mg/dl)	0.11 (0.19)	0.05 (0.10)
Systolic blood pressure (mmHg)	124 (20)	126 (10)
Diastolic blood pressure (mmHg)	78 (10)	76 (10)
Heart rate (bpm)	62 (10)	64 (13)

Data are median (interquartile range) unless otherwise indicated. *$P < 0.01$ vs. low-frequency donors.

120, 150, and 180 min after oral glucose administration. The composite whole-body insulin sensitivity index (WBISI) and the homeostatis model assessment of insulin resistance (HOMA-IR) were calculated as previously described (9,10). Insulin and glucose levels were evaluated by calculating the area under the curve of the incremental insulin and glucose levels by the trapezoidal method. The glucose levels at 2 h after oral glucose loading were also analyzed categorically to determine normal versus abnormal results according to American Diabetes Association criteria (11).

Body composition

Body composition in grams of fat, lean body mass, bone mineral content, and bone mineral density was measured with dual-energy X-ray absorptiometry (Hologic QDR 4500; Hologic, Bedford, MA). The whole-body composition of fat mass, lean body mass, and bone mineral content was measured and reported as subtotals (excluding the contribution from the head of the subject).

Analytical methods

Complete blood cell count (Abbott Celldyne 3500 cell counter), serum iron and total iron binding capacity (Roche Diagnostics Modular P system), percentage of 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 (YSI 2700 STAT Analyzer; Yellow Springs Instruments), insulin levels (radioimmunoassay, Linco Laboratories), and lipid levels (Autanalyzer, model no. 747-202; Roche-Hitachi) were measured in the Yale General Clinical Research Center Laboratory. Plasma C-reactive protein was measured with high-sensitivity C-reactive protein reagent (Alfa Wassermann ACE Clinical System). Serum interleukin-6 was measured with a commercially available enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN).

Ultrasound imaging. Flow-mediated endothelium-dependent vasodilation in the brachial artery was determined with a duplex ultrasound imaging system connected to an 11-MHz transducer by a single blinded investigator, adapted from published guidelines as previously described (3,12). Brachial artery diameter and Doppler blood flow velocity were measured at rest and after transient arterial occlusion induced by 5-min inflation of a forearm pneumatic cuff to 200 mmHg. Flow-mediated dilation was calculated as the percentage of increase in brachial artery diameter 60 s after release of the occluding cuff.

Data analysis

All values are expressed as median (interquartile range). WBISI was the prespecified primary end point for the study. Nonparamateric tests were used to determine differences between low- and high-frequency donors for WBISI and other variables of interest (version 8.0; STATA, College Park, TX; command rank sum). Univariate and multivariate linear regression was used to detect association between predictor variables related to body composition and iron stores to WBISI. Variables with deviation from the normal distribution were log transformed before inclusion in regression models. Based on previous data in nondiabetic obese subjects, we anticipated that the mean ± SD of WBISI in low-frequency blood donors would be 1.8 ± 0.7 units (9). Twenty-one subjects in each group (high- vs. low-frequency donors) provided >80% power to detect a clinically relevant 25% difference (absolute 0.5 index units) between groups with two-tailed $\alpha = 0.05$.

Table 2—*Blood markers of iron status by blood donation frequency*

	Low-frequency donors	High-frequency donors
n	21	21
Serum ferritin (ng/ml)	36 (39)	23 (30)*
Serum iron (mg/dl)	82 (47)	78 (41)
Total iron binding capacity (μg/dl)	322 (37)	326 (76)
Iron binding saturation (%)	26 (9)	21 (12)
Hemoglobin (g/dl)	13.7 (1)	13.3 (2)
Hematocrit (%)	41 (4)	41 (5)
MCV (fl)	91 (4)	91 (7)
MCHC (%)	32 (3)	32 (2)
RDW	11.8 (0.5)	12.2 (0.4)
WBC count (×1,000 cells/mm^3)	5.6 (1.4)	4.8 (1.4)*
Platelet count (×1,000 cells/mm^3)	257 (68)	268 (56)

Data are median (interquartile range) unless otherwise indicated. *$P = 0.05$ vs. low-frequency donors. MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RDW, red cell distribution width; WBC, white blood cell.

Table 3—*Oral glucose tolerance test findings by blood donation frequency*

	Low-frequency donors	High-frequency donors
n	21	21
WBISI	3.7 (2.4)	3.4 (1.5)
HOMA-IR	2.4 (1.7)	2.1 (1.4)
Fasting glucose (mg/dl)	97 (12)	97 (8)
2-h glucose (mg/dl)	126 (36)	120 (49)
3-h glucose AUC (mg × h/dl)	91 (43)	88 (61)
Fasting insulin (μIU/ml)	10 (8)	9 (5)
2-h insulin (μIU/ml)	55 (47)	56 (55)
3-h insulin AUC (μIU × h/ml)	110 (139)	134 (100)
Abnormal OGTT (*n*)*	6	7

Data are median (interquartile range) unless otherwise indicated. *Based on glucose values at 2-h post–oral glucose loading according to American Diabetes Association criteria. AUC, incremental area under curve; OGTT, oral glucose tolerance test.

RESULTS

Clinical characteristics

Clinical characteristics (Table 1) of the high- and low-frequency blood donors did not differ. Only one subject in the study sample (a female high-frequency donor) met diagnostic criteria for the metabolic syndrome.

Laboratory markers of iron stores

Serum ferritin levels and white blood cell count were decreased in high-frequency donors compared with those in low-frequency donors (both $P = 0.05$) (Table 2). Other markers of iron status, hematocrit, hemoglobin, and other measures in the complete blood count did not differ between groups.

Oral glucose tolerance test results

WBISI and HOMA-IR did not differ between high- and low-frequency blood donors ($P = 0.76$ and $P = 0.16$, respectively) (Table 3). Other measures listed in Table 3 also did not differ between groups. In univariate regression analysis, the natural logarithm of WBISI was significantly associated with BMI ($r^2 = 0.21$, $P = 0.002$), total fat mass ($r^2 = 0.14$, $P = 0.02$), waist circumference ($r^2 = 0.13$, $P = 0.02$), C-reactive protein levels ($r^2 = 0.18$, $P = 0.006$), and serum interleukin-6 levels ($r^2 = 0.30$, $P < 0.001$) but was not significantly associated with the natural logarithm of serum ferritin or the number of blood donations over 2 or 10 years. Comparable associations were observed between HOMA-IR and these predictor variables. Adjustment for these predictor variables in multivariate analysis did not alter the univariate finding of no significant association between blood donation frequency and either WBISI or HOMA-IR. In a post hoc analysis, the association between WBISI and blood donation frequency did not differ in subjects with abnormal versus normal glucose tolerance test results (WBISI in abnormal glucose tolerant low-frequency donors 3.9 ± 3.2 vs. high-frequency donors 4.6 ± 3.8; WBISI in normal glucose tolerant low-frequency donors 4.8 ± 2.6 vs. high-frequency donors 4.9 ± 1.9; $P = 0.28$ for glucose tolerance–by–donation frequency interaction).

Brachial artery flow-mediated dilation

Brachial artery flow-mediated dilation (Fig. 1) was greater in high-frequency donors compared with that in low-frequency donors before and during oral glucose tolerance testing (before testing 5.9 ± 4.4 vs. 5.3 ± 2.0%, 1-h post–glucose load 5.0 ± 4.6 vs. 3.9 ± 3.4%, and 3-h post–glucose load 6.1 ± 3.2 vs. 5.1%, respectively; repeated-measures ANOVA $P = 0.045$). The decrease in flow-mediated dilation at 1-h post–glucose loading was significant in both groups (within-group comparisons $P < 0.02$). However, the magnitude of decrease in flow-mediated dilation at 1 h did not differ between high- and low-frequency donors (repeated-measures frequency-by-time interaction term $P = 0.98$). Flow-mediated dilation in the brachial artery was not significantly associated with BMI, total fat mass, waist circumference, or C-reactive protein levels.

CONCLUSIONS — High-frequency blood donation was associated with reduced serum ferritin and increased brachial artery flow-mediated dilation compared with low-frequency blood do-

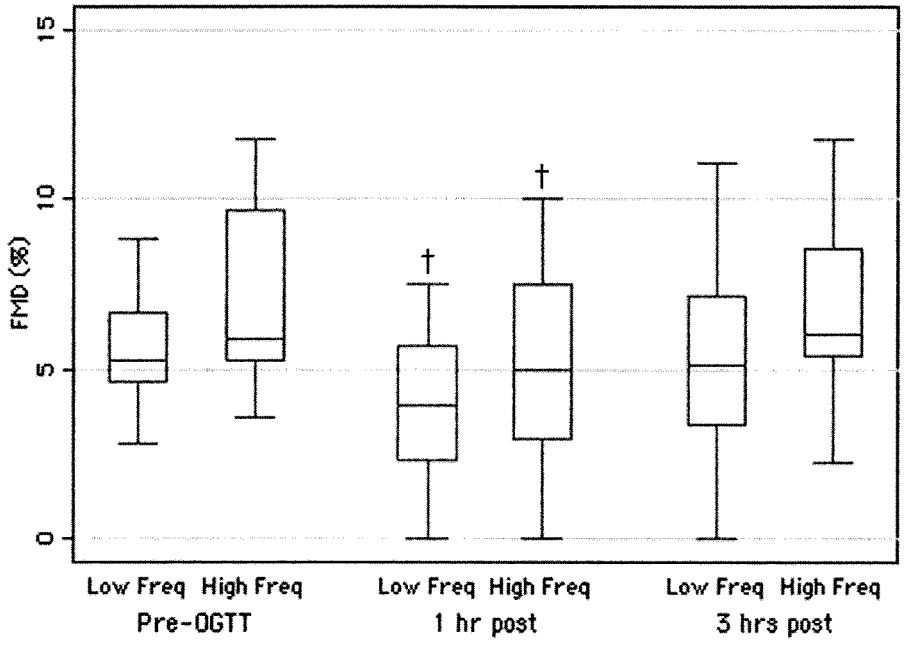

Figure 1—*Flow-mediated dilation (FMD in low-frequency [Low Freq] and high-frequency [High Freq]) blood donors before oral glucose tolerance testing (Pre-OGTT) and at 1 h and 3 h after oral glucose loading. Box plots indicate median value (within box), interquartile range (upper and lower limits of box), and values adjacent to 1.5 × interquartile range (whiskers). Data points outside of whiskers not shown. Flow-mediated dilation in the brachial artery was greater in high-frequency donors compared with that in low-frequency donors before and during oral glucose tolerance testing; repeated-measures ANOVA P = 0.045. †P < 0.02 for within-group comparisons versus pre–oral glucose tolerance testing value.*

nation. High-frequency blood donation did not improve insulin sensitivity after oral glucose loading and did not protect the vascular endothelium from the adverse effects of acute hyperglycemia after oral glucose loading compared with low-frequency blood donation. These findings indicate that increased flow-mediated dilation in high-frequency blood donors is not attributable to improved glucose tolerance.

Severe iron overload in the setting of hereditary hemochromatosis or thalassemia is associated with increased risk of diabetes related to impairment of insulin secretion and/or increased insulin resistance (5,6). In populations with severe iron overload, reduction of body iron stores with either phlebotomy or chelation therapy is associated with improved glucose tolerance (5,6). In subjects without overt iron overload, higher serum ferritin levels are significantly associated with biochemical evidence of decreased insulin sensitivity (5,6). However, epidemiological studies of the association between dietary iron intake or biomarkers of tissue iron stores and the risk of diabetes in populations without overt iron overload have yielded mixed findings (5, 6,13–16). The inconsistencies in these reports may be partly attributable to the presence of inflammation or other unmeasured confounders that alter the relationship between dietary iron intake, biochemical markers of tissue iron stores, and metabolic effects of iron.

In healthy men, a single 500-ml whole-blood donation results in a substantial loss of heme iron (~200–250 mg) and decreases serum ferritin levels by 44% (17). Previous studies on the relationship between blood donation and glucose metabolism have yielded mixed findings. In the Physician's Health Study sample of 33,541 men, blood donation frequency (determined by subject recall) was not associated with risk of incident type 2 diabetes over a 12-year follow-up period (18). Serum ferritin levels (determined in a subset of subjects) ranged from 187 ng/ml in those with no previous history of blood donation to 64 ng/ml in those with a history of ≥30 blood donations. In a cross-sectional study of the effects of blood donation on insulin sensitivity, frequent blood donors (8 subjects with a median of four donations in the past 5 years and mean serum ferritin 87 ng/ml) demonstrated improved insulin sensitivity and decreased insulin secretion during an intravenous glucose tolerance test when compared with sporadic blood donors (13 subjects with one blood donation in the past 5 years and mean serum ferritin 110 ng/ml) and 66 nondonor control subjects (mean serum ferritin 162 ng/ml) (19). In an unblinded and uncontrolled study of 31 subjects with glucose intolerance, serial phlebotomy individually adjusted to induce near iron deficiency (mean serum ferritin decreased from 272 to 14 ng/ml) was associated with evidence of improved insulin sensitivity in response to oral glucose loading and reduced A1C levels (20). In another unblinded, randomized study in 28 patients with diabetes and baseline serum ferritin >200 ng/ml, three serial 500-ml phlebotomies reduced mean serum ferritin from 460 to 232 ng/ml and reduced A1C levels (21). The current study findings add to existing data, as our study subjects had a verified blood donation history (to eliminate recall bias), a greater number of blood donations, and lower serum ferritin levels compared with subjects in most previous reports. In our study sample with severe reductions in iron stores, insulin sensitivity was not associated with serum ferritin levels or the number of blood donations but was significantly associated with known clinical correlates of glucose intolerance related to increased adiposity and blood markers of inflammation. A large proportion of both groups was either overweight or obese, and approximately one-third of our subjects had abnormal glucose tolerance test findings despite very low serum ferritin levels. Taken together, the current findings in blood donors with very low serum ferritin levels and past studies in subjects with and without overt iron overload suggest a possible curvilinear or threshold relationship between serum ferritin and insulin sensitivity, with a relatively steeper slope at high serum ferritin levels and a less steep slope at low serum ferritin levels. Because flow-mediated dilation was significantly greater in high-frequency donors than in low-frequency donors, our findings also suggest differential effects of iron on insulin sensitivity and endothelial function at the lower range of iron storage.

Vascular endothelial dysfunction is associated with atherosclerosis progression and increased risk of morbidity and mortality in cardiovascular disease populations (22). The increased risk of cardiovascular events in diabetic patients is thought to be partly attributable to vascular endothelial dysfunction caused by altered glucose and insulin metabolism (8,23). Our finding of a transient decrease in flow-mediated dilation after oral glucose loading is in accord with previous studies in healthy subjects and in patients with impaired glucose tolerance or diabetes (24,25). Transient attenuation of endothelium-dependent vasodilation is thought to be mediated primarily by acute changes in glucose levels, as suppression of insulin secretion with octreotide does not alter the vascular response to acute hyperglycemia (26). Glucose-induced changes in endothelium-dependent vasodilation may be mediated by increased oxidative stress, as pretreatment with superoxide dismutase, antioxidant vitamins, and tetrahydrobiopterin have been reported to protect against hyperglycemia-induced vascular dysfunction in isolated blood vessels and the intact human circulation (25,27–29). Our observation that the decrease in flow-mediated dilation during oral glucose tolerance testing did not differ between high- and low-frequency blood donors raises the possibility that iron-dependent hydroxyl radical formation may not contribute to hyperglycemia-induced vascular dysfunction in nondiabetic subjects. Alternatively, our findings suggest that the effects of blood donation on insulin sensitivity may differ in subjects with normal versus abnormal glucose tolerance. This interpretation is consistent with the findings of Hirai et al. (30), who demonstrated differential effects of the antioxidant compound vitamin C in subjects with normal versus abnormal glucose tolerance. However, the findings of our post hoc analysis must be interpreted with caution, as the number of subjects in each subgroup is small. Additional studies with an iron chelation agent such as dexrazoxane are warranted to further characterize the relationship among iron-dependent generation of reactive oxygen species, glucose tolerance, and endothelial function (31).

The cross-sectional study design does not provide insight into potential mechanisms that may have contributed to our findings. Although we attempted to carefully characterize our study sample with regard to clinical factors that could influence insulin sensitivity, it is possible that unmeasured confounders may have contributed to the lack of association between blood donation frequency and glucose tolerance. Our findings are also limited to observations during oral glucose loading. Although the WBISI has previously been shown to be significantly associated with

measures of insulin sensitivity obtained with euglycemic insulin clamp techniques (9,10), it is possible that subtle differences in insulin sensitivity between groups were not detectable with our chosen methodology. Finally, the relationship between iron stores and insulin sensitivity observed in our nondiabetic subjects may differ from that in patients with diabetes.

In conclusion, we found no evidence of a significant association among blood donation frequency, serum ferritin levels, and insulin sensitivity during oral glucose tolerance testing in voluntary blood donors. These findings indicate that the mechanisms linking blood donation to improved vascular function are not likely related to changes in glucose metabolism.

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