Clinical Chemistry 51:7
1201–1205 (2005)

Endocrinology and Metabolism

Iron Stores, Blood Donation, and Insulin Sensitivity and Secretion

José Manuel Fernández-Real,* Abel López-Bermejo, and Wifredo Ricart

Background: **Epidemiologists have observed that blood donation is associated with decreased risk of type 2 diabetes and cardiovascular disease.**

Methods: **We investigated the relationship between iron stores and insulin sensitivity, after controlling for known confounding factors, and compared insulin sensitivity between blood donors and individuals who had never donated blood (nondonors). In 181 men, insulin sensitivity and insulin secretion were evaluated through frequently sampled intravenous glucose tolerance tests with minimal model analysis. Men who donated blood between 6 months and 5 years before inclusion (n = 21) were carefully matched with nondonors (n = 66) for age, body mass index, waist-to-hip ratio, and cardiovascular risk profile, including blood lipids, blood pressure, and smoking status.**

Results: **Frequent blood donors (2–10 donations) had increased insulin sensitivity [3.42 (1.03) vs 2.45 (1.2) × $10^{-4} \cdot min^{-1} \cdot$ mIU/L; $P = 0.04$], decreased insulin secretion [186 (82) vs 401.7 (254) mIU/L · min; $P < 0.0001$], and significantly lower iron stores [serum ferritin, 101.5 (74) vs 162 (100) μg/L; $P = 0.017$] than nondonors, but the 2 groups had similar blood hematocrits and blood hemoglobin concentrations.**

Conclusions: **Blood donation is simultaneously associated with increased insulin sensitivity and decreased iron stores. Stored iron seems to impact negatively on insulin action even in healthy people, and not just in classic pathologic conditions associated with iron overload (hemochromatosis and hemosiderosis). According to these observations, it is imperative that a definition of excessive iron stores in healthy people be formulated.**

Section of Diabetes, Endocrinology and Nutrition, University Hospital of Girona "Dr Josep Trueta", Girona, Spain.

*Address correspondence to this author at: Unit of Diabetes, Endocrinology and Nutrition., Hospital de Girona "Dr Josep Trueta", Ctra. França s/n, 17007 Girona, Spain. Fax 34-972-940270; e-mail uden.jmfernandezreal@htrueta.scs.es.

Received December 15, 2004; accepted April 15, 2005.

Previously published online at DOI: 10.1373/clinchem.2004.046847

Iron chelating agents and blood donation can prevent the development of diabetes in transfusional iron overload *(1, 2)*. In apparently healthy people, frequent blood donation leading to decreasing iron stores has been demonstrated to be a protective factor for the development of diabetes mellitus *(3)*. In fact, the higher the ferritin concentrations, the higher the incidence of type 2 diabetes, according to epidemiologic studies *(4, 5)*. These observations are particularly important given the high prevalence of increased iron stores in the general populations of Western countries *(6)*.

Recent randomized studies suggest that iron stores influence insulin action in type 2 diabetes. A statistically significant increase in insulin sensitivity was observed in patients who underwent blood letting [3 extractions of 450 g (500 mL of blood) during a 4-month period] *(7)*. In patients with nonalcoholic steatohepatitis, blood donation also led to decreased insulin concentrations *(8)*. Facchini and Saylor *(9)* have also reported that a low-iron diet positively influences cardiovascular risk in type 2 diabetes.

We hypothesized that insulin sensitivity would increase as iron stores decreased in nonpathologic conditions. Therefore, we studied insulin sensitivity in healthy blood donors compared with a control group who had never given blood (nondonors) matched for sex, age, body mass index (BMI), waist-to-hip ratio (WHR), glucose tolerance, and cardiovascular risk profile.

Materials and Methods

PARTICIPANTS

A total of 181 consecutive healthy men fulfilling the inclusion criteria and enrolled in a cross-sectional, population-based study in Northern Spain were studied. To exclude confounding variables, the participants were asked about blood donations in the previous 5 years and were included in the study only if the last donation had been given more than 6 months before evaluation.

All participants were of Caucasian origin and reported that their body weight had been stable for at least 3 months before the study. None of the patients was taking any medication or had any evidence of metabolic disease

other than obesity. Type 2 diabetes and glucose intolerance were ruled out by an oral glucose tolerance test.

Inclusion criteria were as follows: (*a*) BMI <40 kg/m^2, (*b*) absence of any systemic disease, and (*c*) alcohol intake <40 g a day. Exclusion criteria included the following: (*a*) serum liver enzyme (aspartate aminotransferase and alanine aminotransferase) activities above the upper limits of the reference intervals; (*b*) increased serum creatinine; (*c*) previous acute major cardiovascular event; (*d*) acute illnesses and current evidence of acute or chronic inflammatory or infectious diseases; (*e*) history of transfusion or iron or vitamin therapies in the previous 5 years; (*f*) history of disturbances in iron balance (e.g., hemosiderosis from any cause, atransferrinemia, paroxysmal nocturnal hemoglobinuria, iron deficiency); and (*g*) mental illness rendering the person unable to understand the nature, scope, and possible consequences of the study. Informed written consent was obtained after the purpose, nature, and potential risks were explained to the subjects. The experimental protocol was approved by the Hospital Ethics Committee.

Blood donors (n = 21) were carefully matched to nondonors for age, BMI, WHR, systolic and diastolic blood pressure, proportion of smokers, blood lipids, and cardiovascular risk factors. In this comparison, given the clinical and anthropometric characteristics of blood donors, we excluded nondonors younger than 35 years (n = 26) or older than 65 years (n = 24), with a BMI <23 kg/m^2 (n = 11) or >35 kg/m^2 (n = 23). The proportions of blood donors (21%) and nondonors (79%) in the individuals excluded were similar to those who were finally compared.

MEASUREMENTS

Each participant was studied in the research laboratory in the postabsorptive state. The room was quiet, lights were dimmed, and the temperature was controlled at 23 °C. BMI was calculated as weight (in kilograms) divided by height (in meters) squared. The waist was measured with a soft tape midway between the lowest rib and the iliac crest, and the hip circumference was measured at the widest part of the gluteal region. The WHR was then calculated. Blood pressure was measured after the person had rested for 10 min in the supine position. A standard sphygmomanometer of appropriate cuff size was used on the individual's right arm while the individuals remained in a supine position, and the first and fifth phases were recorded. Values used in the analysis are the mean of 3 readings taken at 5-min intervals. Patients were requested to abstain from alcohol and caffeine for at least 12 h before the different tests.

INSULIN SENSITIVITY AND SECRETION

Insulin sensitivity was measured on a different day from other measurements by use of the frequently sampled intravenous glucose tolerance test. In brief, the experimental protocol started between 0800 and 0830 after an overnight fast. A butterfly needle was inserted into an antecubital vein, and patency was maintained with a slow saline drip. Basal blood samples were drawn at 30, 10, and 5 min before the glucose injection, after which glucose (300 mg/kg of body weight) was injected over 1 min starting at time 0, and insulin (0.03 IU/kg; Actrapid; Novo) was administered at time 20 min. Additional samples were obtained from a contralateral antecubital vein at times up to 180 min, as described previously *(10)*.

Insulin secretion was calculated as the insulin area during the first 10 min of the frequently sampled intravenous glucose tolerance test.

ANALYTICAL DETERMINATIONS

Serum glucose concentrations were measured in duplicate by the glucose oxidase method on a Beckman Glucose Analyzer II. Hemoglobin A_{1c} was measured by HPLC on a fully automated glycohemoglobin analyzer system (Hitachi L-9100). Serum ferritin was measured by microparticle enzyme immunoassay (AxSYM^{TM}; Abbot Laboratories) with intra- and interassay CVs <6%. Whole-blood hemoglobin concentrations and hematocrits (EDTA sample) were determined by routine laboratory tests (Coulter Electronics).

Total serum cholesterol was measured by the reaction of cholesterol esterase, cholesterol oxidase, and peroxidase on a BM/Hitachi 747. HDL-cholesterol was quantified after precipitation with polyethylene glycol at room temperature. Total serum triglycerides were measured by the reaction of glycerol-phosphate-oxidase and peroxidase.

STATISTICAL METHODS

Descriptive results for continuous variables are reported as the mean (SD). Before statistical analyses, gaussian distribution and homogeneity of the variances were evaluated by use of the Levene test; variables were then log-transformed if necessary. The variables that were log-transformed (triglycerides, insulin sensitivity, and insulin secretion) were analyzed on a log scale and tested for significance on that scale. The anti-log-transformed values of the means are reported in the tables. Relationships between variables were tested by the Pearson test and stepwise multiple linear regression analysis. We used the χ^2 test for comparisons of proportions and unpaired or paired *t*-tests for comparisons of quantitative variables. The analyses were performed with the program SPSS (Ver. 11.0).

Results

All participants (n = 181) were healthy men 46 (13.8) years of age with a BMI of 30.3 (8.5) kg/m^2 and a WHR of 0.93 (0.07). In these individuals, serum ferritin was associated with insulin sensitivity ($r = -0.23$; $P = 0.002$; Fig. 1), and this association remained significant in a multivariate linear regression analysis after we controlled for age, BMI, and WHR ($P = 0.01$). Age, BMI, WHR, and

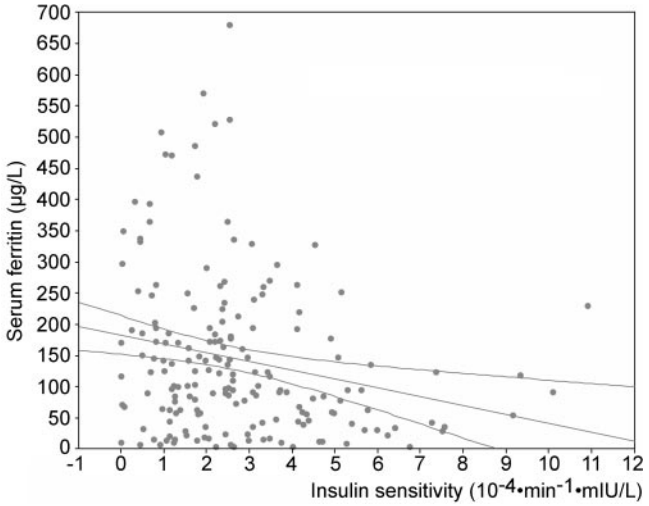

Fig. 1. Linear correlation analysis of the relationship between insulin sensitivity (*x axis*) and serum ferritin concentration (*y axis*) in 181 healthy men.

serum ferritin, included as independent variables, contributed to 40% of the variance in insulin sensitivity.

As stated above, blood donors were carefully matched with nondonors for age, BMI, WHR, systolic and diastolic blood pressure, proportion of smokers, and blood lipids. Blood donors were classified as occasional donors (1 blood donation in the period 6 months to 5 years before the study; group 1) and frequent blood donors (at least 2 blood donations in the same period, median = 4 donations; group 2; Table 1). The number of blood donations correlated with insulin sensitivity ($r = 0.28$; $P = 0.01$; n = 87), and this association was more statistically significant when only blood donors were considered ($r = 0.60$; $P = 0.003$; n = 21). This result was mainly attributable to the men who had given at least 2 blood donations in the previous 6 months to 5 years.

Serum ferritin concentrations were significantly lower among blood donors [101.5 (74) vs 162 (100) µg/L; $P = 0.017$]. In parallel to decreased iron stores, insulin sensitivity was significantly higher [3.42 (1.03) vs 2.45 (1.2) $\times 10^{-4} \cdot min^{-1} \cdot$ mIU/L; $P = 0.04$] and insulin secretion significantly lower [186 (82) vs 401.7 (254) mIU/L · min; $P < 0.0001$], as a reflection of improved insulin sensitivity, among frequent donors (Fig. 2).

Discussion

Our findings support the hypothesis that decreased iron storage through blood donation leads to increased insulin sensitivity, even in healthy individuals. Although those blood donors may be more health-conscious, in this study they were carefully matched for metabolic and cardiovascular risk factors with nondonors.

In patients with type 2 diabetes, blood donation has also been shown to be associated with increased insulin sensitivity *(7)*. The most striking aspect of that observation was the longevity of the effects of blood donation. The changes in insulin sensitivity were maintained even 1 year after the procedure *(7)*. For that reason, we hypothesized that blood donations in the previous 5 years might lead to relatively stable changes in insulin sensitivity. We observed these changes, but only in regular blood donors, defined as those who donated 2 or more times in the previous 5 years. These results are probably attributable to the relatively weaker effects of 1 isolated blood donation. Interestingly, the number of blood donations correlated with insulin sensitivity.

As we have hypothesized *(7)*, the effects of blood donation may be explained by removal of free transition metals from the body, and a substantial time is needed for concentrations to again build up to predonation concentrations. The complex process of advanced glycation end product formation produces reactive oxygen species by

Table 1. Clinical and biochemical values [mean (SD)] for study participants.

		Blood donors		
Variable	Control group	Group 1	Group 2	*P*
No. in group (total n = 87)	66	13	8	
Age, years	51.5 (11.4)	52.5 (10)	56 (13.5)	NS[a]
Smokers, n	3	1	1	NS
BMI, kg/m^2	27.4 (3.6)	27.5 (2.3)	27 (2.6)	NS
WHR	0.93 (0.07)	0.92 (0.04)	0.94 (0.05)	NS
Systolic blood pressure, mmHg	128.2 (14.2)	121.1 (12.1)	121.5 (15.3)	NS
Diastolic blood pressure, mmHg	80 (9.3)	81.9 (9.6)	76.1 (9.1)	NS
Cholesterol, mg/L	2125 (380)	2063 (430)	2245 (314)	NS
Triglycerides, mg/L	1009 (480)	1037 (520)	793 (234)	NS
Fasting glucose, mg/L	960 (100)	943 (53)	1000 (95)	NS
Fasting insulin, mIU/L	9.8 (5.2)	9.5 (3.9)	7.4 (3)	NS
Blood hemoglobin, mg/L	144 (10)	146 (12)	144 (9)	NS
Blood hematocrit, %	42 (3.8)	43 (3.3)	42.7 (2.7)	NS
Serum ferritin, µg/L	162 (100)	110 (62)[b]	86.9 (95)[b]	0.017

[a] NS, not significant.
[b] Compared with the control group.

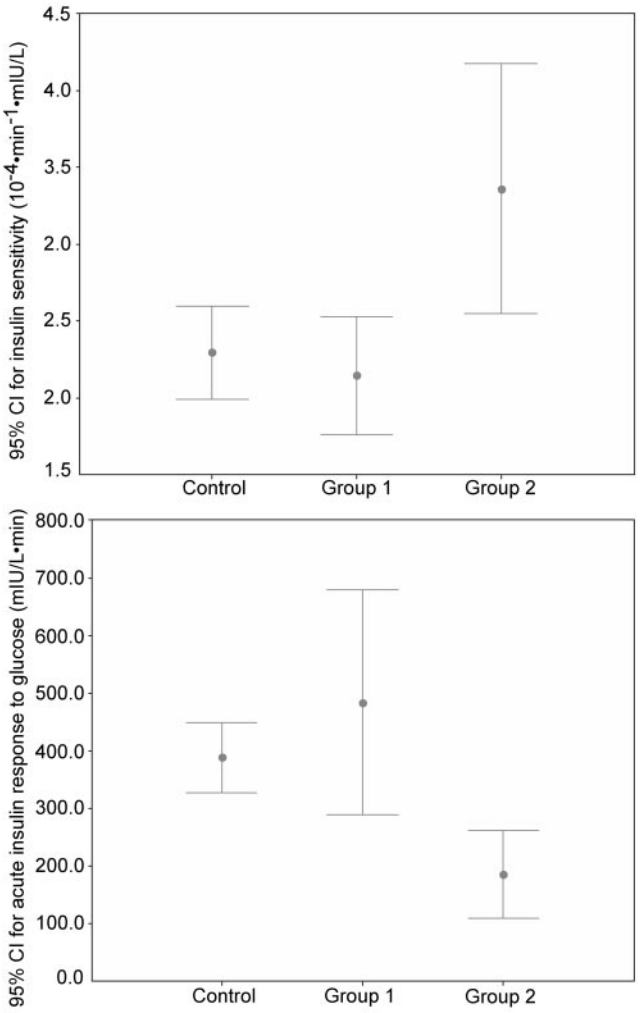

Fig. 2. 95% confidence intervals (*CI*) for insulin sensitivity (*top*) and insulin secretion (*bottom*) in control individuals (nondonors), sporadic donors (*Group 1*), and frequent donors (*Group 2*).

Lower and *upper error bars* indicate the 95% confidence interval for the mean. The difference is statistically significant in group 2 compared with the rest of the groups.

metal-catalyzed reactions. Advanced glycation end products themselves bind transition metals *(11)*, potentiating their toxic effects, including insulin resistance. Reactive oxygen species interfere with insulin signaling at various levels, impairing insulin uptake through a direct effect on insulin receptor function *(12)* and inhibiting the translocation of GLUT4 to the plasma membrane *(13)*. Decreasing iron stores would ameliorate insulin resistance by reducing this cascade of events.

Remarkably, a lower prevalence of diabetes mellitus among frequent blood donors has been reported *(3)*. In that report, no measure of insulin sensitivity was available *(3)*. More recently, dietary iron intake, mainly heme-iron intake from red meat sources, was associated with an increased risk of type 2 diabetes *(14, 15)*. Increased iron stores predicted the development of diabetes in epidemiologic studies *(4, 5)*. Facchini *(16)* found significant reductions in insulin concentrations in healthy volunteers 1 month after they had undergone a 550-mL phlebotomy. It has also been suggested that the increased insulin sensitivity observed in vegetarians might be related to their low–iron-content diet *(17)*.

Iron interferes with insulin inhibition of glucose production by the liver. In fact, a very common abnormality seen in iron overload is insulin resistance. Hepatic extraction and metabolism of insulin decrease with increasing iron stores, leading to peripheral hyperinsulinemia *(18, 19)*. There is some evidence that iron overload also affects skeletal muscle *(20)*, the main effector of insulin action.

The association of serum ferritin with insulin sensitivity could be attributable to extreme values of serum ferritin, reflecting inflammation. However, in this study, after we excluded individuals with serum ferritin >200 μg/L, the association remained statistically significant ($r = -0.20$; $P = 0.016$; n = 144). Inflammation may indeed cause increased serum ferritin concentrations. In this series, the association between insulin sensitivity and serum C-reactive protein concentration was also statistically significant ($r = -0.19$; $P = 0.025$; n = 144; data not shown), but serum C-reactive protein and ferritin were not associated with each other ($r = -0.09$; $P = 0.2$).

The strengths of this study lie in the methodology of evaluating insulin sensitivity and insulin secretion in individuals carefully matched for metabolic and cardiovascular risk factors. However, this is a cross-sectional case–control study, which needs to be replicated in prospective observations. This study confirms previous observations linking insulin sensitivity and serum ferritin concentrations in smaller *(10)* and larger series (n = 202 men) *(21)*, as well as recent epidemiologic observations *(22)*. Other limitations include possible survival bias, although it is probably small, and the failure to determine serum ferritin concentrations and insulin sensitivity before the initial blood donation.

In conclusion, our findings show that a definition of excessive iron stores in healthy people is needed. In addition, blood donation or phlebotomy may be indicated as adequate and safe therapy for prevention of type 2 diabetes among persons with high-normal serum ferritin *(23)*. A trial investigating the role of blood donation or phlebotomy in the long-term improvement of insulin and glucose metabolism should be performed in groups at increased metabolic risk.

This work was supported by research grants from the Instituto de Salud Carlos III (RCMN C03/08, RGDM G03/212, and RGTO G03/028).

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