Blood Donations and Risk of Coronary Heart Disease in Men

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Background—In experimental animals, iron overload appears to promote atherosclerosis and ischemic myocardial damage, but the results of epidemiological studies that relate iron stores to risk of coronary heart disease (CHD) have been inconsistent.

Methods and Results—We prospectively studied blood donations, which effectively reduce body iron stores, in relation to the risk of CHD among participants in the Health Professionals Follow-up Study. The lifetime history of blood donation was assessed with a questionnaire in 1992. The 38 244 men who were free of diagnosed cardiovascular disease at that time were included in the analyses. During 4 years of follow-up, we documented 328 nonfatal myocardial infarctions and 131 coronary deaths. Although the number of lifetime blood donations was strongly associated with lower plasma ferritin levels in a subsample, the blood donation was not associated with risk of myocardial infarction or fatal CHD. The age-adjusted relative risk (RR) of myocardial infarction for men in the highest category of blood donations (≥30) compared with never donors was 1.2 (95% CI 0.8 to 1.8), and this RR was not materially changed after adjustment for several coronary risk factors. No significant associations were found between blood donation and the risk of myocardial infarction in analyses restricted to men with hypercholesterolemia or those who never used antioxidant supplements or aspirin.

Conclusions—The study results do not support the hypothesis that reduced body iron stores lower CHD risk. **(*Circulation*. 2001;103:52-57.)**

Key Words: coronary disease ■ men ■ iron ■ blood donation ■ heart diseases

A role of iron in coronary heart disease (CHD) was proposed by Sullivan[1] in 1981 as an explanation for the sex difference in risk. According to this hypothesis, the loss of iron with menstruation explains the lower risk of CHD in premenopausal women compared with men and postmenopausal women. An adverse effect of iron could be related to its ability to catalyze the formation of highly reactive oxygen species and to promote lipid peroxidation, as shown in vitro.[2] Moreover, in experimental animals, iron overload increases the myocardial damage caused by anoxia and reperfusion,[3] and in 1 study, iron overload increased the atherogenic effects of high-cholesterol diets.[4] Epidemiological investigations that relate body iron stores to the risk of CHD, however, have produced conflicting results[5–8]; these may in part be explained by the use of nonspecific measures of body iron stores, such as serum transferrin.[5] More informative have been investigations that used serum ferritin levels, which is strongly correlated with body iron stores in healthy subjects. However, concerns have been raised because serum ferritin levels increase with inflammation, and there is evidence of an inflammatory component in atherosclerosis.[9] This limitation could be overcome by the use of the history of blood donation as a marker of body iron levels. Because body iron stores in men can be halved through the donation of 1 U blood/y and further reduced to the levels of premenopausal women through the donation of 2 or 3 U/y,[10] the contrast between regular blood donors and nondonors with a similar distribution of coronary risk factors provides a direct and powerful test of the hypothesis that depletion of body iron stores reduces the risk of CHD. This approach was pursued by 2 groups of investigators, who recently reported that blood donors have a lower risk of CHD than nondonors.[11,12] However, in 1 of the studies, information on blood donation was collected after the coronary event[11] and thus was prone to bias; the other study included few blood donors, and the results may have been affected by the inclusion of men with preexisting CHD.[12] We therefore examined the association between blood donation and risk of CHD in the Health Professionals Follow-up Study, a large prospective investigation of US men. Our primary

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hypothesis was that regular blood donation reduces the risk of myocardial infarction.

Methods

The Health Professionals Follow-Up Study

The Health Professionals Follow-up Study began in 1986 when 51 529 health professionals 40 to 75 years old completed a 131-item food-frequency questionnaire[13] and provided information about medical history and heart disease risk factors. Follow-up questionnaires were sent every 2 years. For the present investigation, we used 1992 as the baseline, because that year participants were asked for the first time to report their blood donation history. We excluded from the analysis 10 380 men with prior report (in 1992) of myocardial infarction, angina, coronary artery surgery, stroke, transient ischemic attack, or peripheral arterial disease; 2905 men did not return the 1992 questionnaire. We followed the remaining 38 244 eligible men for coronary disease incidence during the subsequent 4 years. More than 94% returned follow-up questionnaires in each 2-year follow-up cycle. Nonresponding participants not listed in the National Death Index[14] were assumed to be alive.

Assessment of Blood Donations

In the 1992 questionnaire, we asked men to report their total number of blood donations during the past 30 years; possible responses included never, ≤5, 6 to 9, 10 to 19, 20 to 29, 30 to 59, 60 to 89, and ≥90. To validate the self-reported information on the number of blood donations, we measured serum ferritin levels in a random sample of 123 men. Blood samples for the validation study were collected in 1986. Mean ferritin levels according to the number of blood donations were 187 μ/L for no donations; 186 μ/L, 1 to 4; 187 μ/L, 5 to 9; 160 μ/L, 10 to 19; 93 μ/L, 20 to 29; 104 μ/L, 30 to 59; 34 μ/L, 60 to 89; and 43 μ/L, ≥90. Because few men donated >30 U of blood, they were grouped into 1 category (≥30) with a mean ferritin level of 64 μ/L.

Ascertainment of End Points

As described elsewhere in detail,[15] end points were fatal CHD (including sudden death) and nonfatal myocardial infarction; for the present study, we included only events that occurred between the return of the 1992 questionnaire and January 31, 1996. Participants who reported an incident myocardial infarction on a follow-up questionnaire were asked for permission to review medical records. Nonfatal myocardial infarction was confirmed with the use of World Health Organization criteria[16]: symptoms plus either typical ECG changes or elevated cardiac enzymes.

Deaths were reported by next-of-kin, coworkers, or postal authorities or in the National Death Index.[14] Fatal CHD was confirmed with medical records, autopsy reports, or the death certificate if CHD was the underlying cause and a diagnosis of coronary disease was confirmed by other sources. Deaths due to sudden death within 1 hour of the onset of symptoms in men with no other apparent cause of death (other than CHD) were also included.

Statistical Analysis

Participant follow-up time was from the return of the 1992 questionnaire up to the occurrence of an end point, death, or January 31, 1996. Relative risk (RR) was calculated by dividing the incidence of CHD among men in each category of blood donation by the incidence among men who never donated blood. We adjusted RRs for age (5-year categories)[17] and used the Mantel extension test[18] to test for linear trends. To adjust for other risk factors, we used multiple logistic regression. For nondietary risk factors, including the use of vitamin supplements, we used the information provided in the 1992 questionnaire. For dietary risk factors, we used the 1990 questionnaire, because no food frequency questionnaire was administered in 1992. In multivariate models, we evaluated monotonic trends by using the median value of each category and modeling this as a continuous variable. The study had a power of 0.80 to detect a 25% reduction in risk of CHD in blood donors compared with never donors.

Results

During 139 176 person-years of follow-up, we documented 459 events, including 328 nonfatal myocardial infarctions and 131 coronary deaths. Differences in coronary risk factors between blood donors and never donors were modest, except for a lower prevalence of diabetes among blood donors (Table 1). We found virtually no association between the number of blood donations and the risk of myocardial infarction (Table 2). The age-adjusted RR for men in the highest category of blood donations (≥30) compared with never donors was 1.2 (95% CI 0.8 to 1.8) and was not materially changed by further adjustment for other coronary risk factors; the analogous RR for fatal CHD was 1.0 (0.5 to 2.2).

Because previous investigations have suggested that high body iron stores may be particularly deleterious in men with high blood cholesterol concentrations or diabetes,[19] we examined the association between blood donation and risk of CHD among men with these risk factors (Table 3). Men with hypercholesterolemia did not appear to benefit from blood donation; the RR for the top category of donors versus never donors was 1.2 (0.6 to 2.4). The small number of donors among men with diabetes did not allow a precise estimate to be made in this subgroup (RR 0.7, 0.1 to 5.4). Finally, Finnish investigators have hypothesized that the lack of association between iron stores and risk of CHD in US studies may be due to the high frequency of the consumption of antioxidant vitamins or aspirin, which may reduce the deleterious effects of iron.[20] We therefore examined the association between blood donation and the risk of myocardial infarction in our cohort among men who did not take vitamin E or aspirin on a regular basis; we still found no association (Table 3).

Discussion

The present findings do not support the hypothesis proposed by Sullivan[1] in 1981 that depletion of body iron stores reduces the risk of CHD. This hypothesis was originally formulated to explain the low risk of CHD among premenopausal women, who are iron depleted due to menstrual bleeding, and the observation of an increased risk of CHD among women who had undergone hysterectomy without oophorectomy.[1,21] Subsequent observations that the risk of CHD in women who receive postmenopausal estrogen replacement therapy is similar to that of premenopausal women[22] suggested that reduced estrogen levels rather than excess iron account for the increased risk after menopause. A beneficial effect of long-term replacement therapy may, however, be offset by a temporary increase in risk among women with preexisting CHD.[23] The cardiomyopathy of hemochromatosis was also quoted in support of the hypothesis,[1] although there is no evidence of a positive association between hemochromatosis and coronary artery disease.[24] Renewed interest in the possibility that iron is a major risk factor for CHD has followed the 1992 report from Finland of a strong association between serum ferritin levels and the risk of myocardial infarction in men.[19]

TABLE 1. Relation of Selected Risk Factors for CHD to Lifetime Number of Blood Donations

	No. of Blood Donations		
	0 (n=10 735)	10–20 (n=3680)	≥30 (n=1767)
Smokers, %	6.6	6.6	5.8
Body mass index, mean kg/m^2	25.5	26.1	26.2
Hypertension, %	26	26	23
Hypercholesterolemia, %	33	34	30
Diabetes, %	4.1	3.5	2.7
Family history of myocardial infarction, %	11	12	11
Physical activity, mean metabolic Eq/wk	34.7	36.5	37.1
Use of vitamin E supplements, %	21	19	18
Use of iron supplements, %	2.5	2.6	3.3
Use of aspirin, %	29	32	30
Intake* of			
Alcohol, mean g/d	10.2	10.3	10.0
Saturated fatty acids, mean g/d	22.3	23.4	23.7
Folic acid, mean μg/d	507	501	488
Fiber, mean g/d	22.1	21.2	22.2

Values are directly standardized to the age distribution of the entire cohort.

*Estimated with food-frequency questionnaire completed in 1990 and adjusted for total energy intake (except for alcohol).

Iron can catalyze the formation of reactive oxygen species and promote lipid peroxidation in vitro.[2] In addition, the results of animal experiments indicate that iron overload increases myocardial damage caused by anoxia and reperfusion.[3,8] This effect may be related to the generation in the ischemic myocardium of superoxide and hydrogen peroxide, which in the presence of free iron are transformed into highly reactive radicals.[3,5] Furthermore, rabbits experimentally overloaded with iron developed more extensive atherosclerosis when fed high-cholesterol diets.[4] Although these studies support a potential adverse effect of iron, their relevance to human disease remains to be established. In randomized trials among Finnish men, the in vitro resistance of VLDL and LDL to oxidation was modestly increased with three 500-mL blood donations over a course of 14 weeks[25] and decreased with iron sulfate supplementation.[26] On the other hand, attempts to relate iron status to the degree of atherosclerosis measured with Doppler sonography in the carotid artery have produced conflicting results.[27–29] Several investigators attempted to directly relate iron status to the occurrence of CHD. A few years ago, we reviewed these epidemiological studies and concluded that evidence of an adverse effect of iron was unconvincing.[30] Several new studies have been published since then, including 2 prospective investigations that used

TABLE 2. RR of CHD According to Lifetime Number of Blood Donations

		Total Myocardial Infarction			Fatal CHD		
No. of Blood Donations	Person-y	No. of Cases*	Age-Adjusted RR (95% CI)	Multivariate† RR (95% CI)	No. of Cases*	Age-Adjusted RR (95% CI)	Multivariate† RR (95% CI)
0	39 887	148	Reference	Reference	53	Reference	Reference
1–4	41 836	129	0.9 (0.7–1.2)	0.9 (0.7–1.1)	30	0.6 (0.4–1.0)	0.6 (0.4–1.0)
5–9	14 561	46	1.0 (0.7–1.4)	1.0 (0.7–1.4)	9	0.6 (0.3–1.2)	0.6 (0.3–1.2)
10–19	13 778	43	1.0 (0.7–1.4)	1.0 (0.7–1.4)	11	0.8 (0.4–1.5)	0.8 (0.4–1.6)
20–29	6161	21	1.1 (0.7–1.7)	1.1 (0.7–1.7)	9	1.5 (0.7–3.0)	1.6 (0.8–3.2)
≥30	6617	27	1.2 (0.8–1.8)	1.3 (0.8–1.9)	7	1.0 (0.5–2.2)	1.1 (0.5–2.5)
χ, trend	...	...	0.9	1.3		0.07	1.0
P	...	...	0.4	0.2		0.9	0.3

*Forty-five cases (including 12 fatal) that occurred in men who did not report number of blood donations in the 1992 questionnaire are not shown in the table.

†Adjusted for age (5-year groups), body mass index (quintiles), smoking (never, past, current: 1–14, 15–24, ≥30 cigarettes/d), physical activity (quintiles), alcohol intake (g/d: 0, 1–4, 5–9, 10–14, 15–29, ≥30), use of vitamin E supplements, family history of myocardial infarction, and history of diabetes, hypertension, and high blood cholesterol.

TABLE 3. Multivariate RR of MI According to Lifetime Number of Blood Donations in Men With Selected Risk Factors for CHD

	No. of Blood Donations						
	0	1–4	5–9	10–19	20–29	≥30	*P*, Trend
Hypercholesterolemia							
Person-y	8289	9259	3237	3123	1374	1360	...
No. of cases	37	29	7	10	6	6	...
RR	Reference	0.8	0.6	0.9	1.2	1.2	>0.4
95% CI	...	0.6–1.2	0.3–1.1	0.5–1.5	0.6–2.4	0.6–2.4	...
Nonuse of vitamin E supplements							
Person-y	31 465	33 289	11 810	11 206	5102	5457	...
No. of cases	119	100	34	32	19	21	...
RR	Reference	0.9	0.9	0.9	1.2	1.2	>0.3
95% CI	...	0.7–1.1	0.6–1.3	0.6–1.3	0.7–1.9	0.7–1.9	...
Nonuse of aspirin							
Person-y	28 333	29 403	10 007	9426	4273	4566	...
No. of cases	109	96	29	32	11	16	...
RR	Reference	0.9	0.9	1.0	0.8	1.1	>0.9
95% CI	...	0.7–1.2	0.6–1.3	0.7–1.6	0.4–1.5	0.6–1.8	...
Current smoking							
Person-y	1571	1732	601	495	278	202	...
No. of cases	12	8	5	5	1	3	...
RR	Reference	0.5	0.9	1.1	1.5	1.8	0.07
95% CI	...	0.2–1.1	0.4–2.5	0.4–2.7	0.5–4.5	0.6–5.5	...
Diabetes							
Person-y	1313	974	340	300	122	122	...
No. of cases	12	11	3	2	1	1	...
RR	Reference	1.1	0.9	0.6	0.5	0.7	>0.4
95% CI	...	0.5–2.5	0.2–3.2	0.1–2.6	0.1–4.6	0.1–5.4	...

serum ferritin as a measure of iron stores.[7] The use of ferritin is important, because null results obtained in investigations that used serum iron or transferrin saturation may be attributed to their poor correlation with iron stores within the physiological range.[30] Overall, the association between serum ferritin and risk of CHD has been examined in 6 separate cohorts.[7,19,31–34] A significant positive relation was found in 2: the Kuopio Study in Finland[19] and the Bruneck Study in Italy.[33] This last investigation, however, included individuals with prevalent CHD, which may have contributed to the positive findings.

More recently, 2 groups of investigators examined the risk of CHD among heterozygous carriers of the hemochromatosis gene. One of these investigations was conducted among participants in the Kuopio Study. The RR of acute myocardial infarction among carriers of the hemochromatosis gene (76 heterozygous and 1 homozygous) compared with noncarriers was 2.3 (95% CI 1.1 to 4.8; $P=0.03$).[35] The second investigation was conducted in the Netherlands among 12 239 women 51 to 69 years of age who were followed for 16 to 18 years.[36] The RR for fatal myocardial infarction among women who carried the hemochromatosis gene compared with noncarriers was 1.5 (95% CI, 0.9 to 2.5; $P=0.14$). Although these data are consistent with the hypothesis that iron is a risk factor for CHD,[37] there are alternative explanations for these findings. The hemochromatosis gene is in the major histocompatibility complex region of chromosome 6. Several genes in this region are highly polymorphic and encode proteins involved in immune and inflammatory responses. Thus, the reported associations could be due to linkage disequilibrium between the hemochromatosis gene and other polymorphic genes. Most importantly, even if a specific association between the hemochromatosis gene and risk of CHD were to be confirmed, it would still not imply that the increased risk is related to the iron accumulation itself rather than to other metabolic effects. Therefore, a direct assessment of the role of iron stores in the risk of CHD remains necessary.

The comparison of blood donors with nondonors appears to provide a strong test of the iron hypothesis, because of the marked contrast in body iron stores of regular donors compared with those of nondonors.[10] Although it may be expected that blood donors are on average healthier than nondonors, the fact that we found little difference in the major coronary risk factors between the 2 groups in this relatively homogeneous cohort of health professionals and the similarity be-

tween age-adjusted and multivariate RRs in our analyses suggest that confounding is modest in these data. Moreover, confounding would be more likely to cause an overestimation than an underestimation of the protective effects of blood donation. Attenuation of any association between blood donation and risk of CHD could have occurred if there was substantial error in the self-reported number of blood donations. However, the 3-fold variation in serum ferritin levels that we found in the validation study between frequent donors and never donors suggests that any such attenuation would have been modest. Finally, the high response rates minimized bias from losses to follow-up. A 6% loss to follow-up is among the lowest in prospective studies, and potential for bias is minimal except under the most unusual circumstances. Thus, the lack of association between a history of blood donation and the risk of CHD in this large cohort provides strong evidence against the hypothesis that iron depletion reduces coronary risk among healthy US men.

The association between blood donation and risk of coronary events was previously examined in 2 investigations: 1 in Nebraska[11] and 1 in Kuopio, Finland.[12] In the Nebraska study, blood donors had half the risk of cardiovascular events than nondonors in crude analyses, but this difference was attenuated and no longer significant after adjustment for potential confounders. Moreover, this residual association is likely to have been spurious, because the history of blood donation was obtained retrospectively via telephone at the end of the follow-up, and the fact that the occurrence of a cardiovascular event may have changed the blood donation habits was ignored. On the other hand, in the Kuopio Study, men who donated blood in the 2 years before the baseline had a markedly reduced risk of CHD compared with nondonors during the 8 years of follow-up, and the difference remained significant after adjustment for potential confounders (RR 0.12, 95% CI 0.02 to 0.86).

One of the differences between our study and the Finnish study is that the Kuopio cohort included men with a history of CHD at baseline. Analyses were adjusted for the presence or absence of coronary history that, as expected, was much more common among nondonors but not for the severity of the disease, so some residual confounding is likely. It seems unlikely, however, that this could entirely explain the strong inverse association that was reported. In addition, there are the marked differences between the participants in each study, such as the lower prevalence of smoking, higher use of antioxidant vitamins, and lower mean cholesterol levels in the US health professionals compared with the Finnish men. Differences in cholesterol levels and use of antioxidant vitamins do not appear to explain the discordant results, because we found no association between blood donation and risk of CHD among men with history of high cholesterol or nonusers of vitamin E or multiple vitamins. However, there were too few current smokers or diabetics in our cohort to obtain stable RR estimates within these groups; therefore, we cannot exclude the possibility that they may benefit from blood donation. Also, we cannot exclude the possibility that a frequency of blood donation sufficiently high to cause a substantial reduction in hematocrit could reduce the risk of CHD. A modest but significant association between hematocrit and risk of CHD was shown in a recent meta-analysis of prospective studies.[38]

In summary, the results of our study suggest that body iron stores are not a major coronary risk factor among US men without previous cardiovascular disease or diabetes. This conclusion is consistent with previous prospective investigations that found no association between serum ferritin and risk of CHD.

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