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Donation of Blood Is Associated with Reduced Risk of Myocardial Infarction

The Kuopio Ischaemic Heart Disease Risk Factor Study

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Because high body iron stores have been suggested as a risk factor for acute myocardial infarction, donation of blood could theoretically reduce the risk by lowering body iron stores. For this reason, the authors tested the hypothesis that voluntary blood donation is associated with reduced risk of acute myocardial infarction in a prospective epidemiologic follow-up study in men from eastern Finland. The subjects are all participants of the Kuopio Ischaemic Heart Disease Risk Factor Study. A cohort of 2,862 men aged 42–60 years were followed for an average of almost 9 years. One man (0.7%) out of 153 men who had donated blood in 24 months preceding the baseline examination experienced an acute myocardial infarction during 1984 to 1995, whereas 316 men (12.5%) of 2,529 non-blood donors had an acute myocardial infarction ($p < 0.0001$ for difference between proportions). In a Cox proportional hazards model adjusting for age, examination years and all other predictive coronary disease risk factors, blood donors had a 88% reduced risk (relative hazard = 0.12, 95% confidence interval 0.02–0.86, $p = 0.035$) of acute myocardial infarction, compared with non-blood donors. These findings suggest that frequent blood loss through voluntary blood donations may be associated with a reduced risk of acute myocardial infarction in middle-aged men. *Am J Epidemiol* 1998;148:445–51.

cardiovascular diseases; iron; myocardial infarction; population studies; risk factors

Premenopausal women have lower incidence of and lower mortality from coronary heart disease compared with men of similar age or with postmenopausal women (1, 2). It has been hypothesized (1) that this divergence is not solely explained by hormonal differences but also by frequent iron loss through menstruation. In accordance with this hypothesis, some studies (3, 4) show that the risk of coronary disease in women is increased by simple hysterectomy without oophorectomy, which suggests that menstrual bleeding might play an important role in the risk reduction.

Iron is a transition metal that can catalyze toxic redox reactions, and it has been suggested that it is involved in many harmful biologic processes and in disease development in the human body (5–7). We have proposed that excessive iron is a potent risk factor for acute myocardial infarction (8–11). Supporting evidence comes from in vitro lipid peroxidation and lipoprotein modification studies (12, 13), from cholesterol fed iron overloaded animal models (14, 15), and from analyses of the composition of human atherosclerotic lesions (16, 17). The evidence from prospective human population studies is inconsistent (10, 11, 18–20). Increased estimated body iron stores have been associated with increased risk of coronary disease death or acute myocardial infarction in some studies (8, 9, 21), but not in all studies (22–26). The discrepancy is most likely due to both biologic and measurement variability in assessing the body iron stores and study outcomes (9–11, 18). In US cohorts, a large proportion of the subjects used asetosalisylic acid or fish oil preparations, which induce blood loss and could thus confound the relation between iron status measurements and the risk of coronary disease. In addition, in the United States, the use of antioxidant supplements is common. It has been theorized that antioxidants attenuate iron overload's deleterious effects, which are mediated through enhanced lipid peroxidation in both the arterial wall and the cardiac muscle (10, 11).

The role of blood loss and consequent decrease in body iron on the risk of acute myocardial infarction and coronary disease can be studied by comparing voluntary blood donors to non-blood donors. In each

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Abbreviations: AMI, acute myocardial infarction; HDL, high density lipoprotein cholesterol; KIHD, Kuopio Ischaemic Heart Disease Risk Factor Study; LDL, low density lipoprotein cholesterol.

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donation of 500 ml of whole blood, around 225–250 mg of heme iron is removed. We have studied the association of blood donations with the risk of acute myocardial infarction in the Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD). The preliminary findings showed that men who had donated blood regularly had a greatly reduced risk of acute myocardial infarction compared with non-blood donors (27). Our first short report was based on a short follow-up and thus the statistical power did not enable a thorough analysis of confounding factors. Similar findings were recently reported from a cohort study in Kansas (28). That study did not have comprehensive measurements of risk factors. Because blood donors are more healthy and health conscious than non-blood donors (27, 28), the observed reduced risk of coronary disease could be due to a self-selection bias. For this reason, we carried out an exhaustive analysis of possible confounding factors in the association between donating blood and the risk of acute myocardial infarction. The present paper presents a detailed analysis of confounding, based on an extended follow-up of myocardial infarctions.

MATERIALS AND METHODS

Study population

The KIHD study is a population-based study of previously unestablished and traditional risk factors for cardiovascular diseases, atherosclerosis, and related outcomes among middle-aged men from the Kuopio region in eastern Finland (29), an area with particularly high incidence of and mortality from coronary disease (30). The baseline examinations were carried out between March 1984 and December 1989. The study sample included 3,235 men aged 42, 48, 54, or 60 years at the baseline examination. Of these men, 2,682 subjects (82.9 percent) participated.

Examination protocol

Venous blood samples were drawn from vena basilica with Venoject vacuum tubes (Terumo Corp., Tokyo, Japan). The subjects were instructed to abstain from alcohol for 3 days, from smoking for 12 hours, and from eating for 12 hours. Samples were drawn between 8:00 a.m. and 10:00 a.m. The examination protocol and measurements have been described in detail previously (8, 27, 31–38).

Blood donations

Information about blood donations in the study cohort was obtained by data linkage from the records of the local Red Cross office, the only site where residents of the catchment area donate blood. Permission for the data linkage was obtained from the Finnish Red Cross. Data on blood donations during 1982 to 1989 were collected in 1990. A dichotomous variable was constructed that indicated whether the subject had donated blood in the 24 months preceding the baseline examination (yes vs. no). Less frequent blood donations were considered unimportant. In each donation, 450 g (500 ml) of blood is taken by venesection (phlebotomy).

Assessment of risk factors

History of myocardial infarction, angina pectoris and other ischemic heart disease, the presence of hypertension, current antihypertensive medication and history of smoking were recorded using a self-administered questionnaire, which was checked by an interviewer (29). Re-interviews to obtain medical history were conducted by a physician.

The life-long exposure to smoking, cigarette-years, was estimated as the product of years smoked and the number of tobacco products (cigarettes, cigars or pipefuls) smoked daily at the time of examination. The consumption of foods was assessed with an nutritionist-instructed 4-day food recording by household measures (39). The intake of nutrients was estimated using the Finnish NUTRICA software (Social Insurance Institution, Helsinki). Resting blood pressure was measured between 8:00 a.m. and 10:00 a.m. with a random-zero mercury sphygmomanometer by a protocol previously described (8, 31, 32).

The family history of coronary disease was defined positive if one or more of biologic parents or siblings had coronary disease history. Coronary disease in the subject was defined positive if there was history of or prevalent coronary disease. History of coronary disease was positive if there was medical history of myocardial infarction or angina pectoris, or if there was angina pectoris on effort in the London School of Hygiene interview (40). Prevalent coronary disease was positive if there was evidence of prevalent coronary disease during a maximal symptom-limited exercise test or if the person used nitroglycerin tablets once or more per week. The criteria for coronary disease during the exercise test have been previously described (32).

High density lipoprotein (HDL) 2 subfraction was separated from unfrozen serum by ultracentrifugation, and cholesterol was measured enzymatically (31). Serum concentration of apolipoprotein B was measured immunoturbidometrically (KONE Oy, Espoo, Finland) using antiserum from Orion (Espoo, Finland) (8, 31). Apolipoprotein B was used as a covariate instead of low density lipoprotein (LDL), because it is a

stronger predictor of acute myocardial infarction (31). Blood glucose was measured by a glucose dehydrogenase method after precipitation of the proteins with trichloric acetic acid. Diabetes mellitus was defined as blood glucose ≥6.7 mmol/liter or previously diagnosed diabetes with treatment. Plasma fibrinogen concentration was measured in fresh samples using a functional measurement (33). Mercury in hair and urine samples was determined by flow injection analysis-cold vapor atomic absorption spectrometry and amalgamation (34). Serum selenium was measured by an atomic absorption spectrometric method (34). Plasma ascorbate concentrations were measured with a high-performance liquid chromatography method (35). A large number of both social and psychological characteristics of the participants were assessed by self-administered questionnaires (33, 36–38).

Myocardial infarction

Acute myocardial infarctions (AMIs) among the study cohort members that occurred between March 1984 and December 1992 were registered as part of the multinational MONICA (Monitoring of Trends and Determinants in Cardiovascular Disease) Project (41). The registry collected detailed diagnostic information on all ischemic heart attacks in the population in a prospective manner. Events were classified either as definite AMI, possible AMI, prolonged chest pain, no AMI, or insufficient data, according to uniform predefined diagnostic criteria based on symptoms, electrocardiogram findings, and cardiospecific enzymes. Data on myocardial infarctions between January 1993 and December 1995 were obtained from the national hospital discharge registry. Because we used the national social security code to track the men, there were no losses to follow-up. The diagnostic classification was carried out by one internist (TAL) using identical procedures and criteria to those used in the MONICA Project. A total of 317 of the 2,682 men at risk had a fatal or nonfatal AMI between March 1984 and December 1995. The follow-up period for individual subjects was up to 10 3/4 years, and the mean follow-up time was approximately 8 1/2 years.

Statistical methods

All data analyses were done using SPSS-X statistical software (SPSS Inc., Chicago, Illinois) for IBM RS6000 work-station (IBM Corporation, Armonk, New York). The statistical significance of differences between means were estimated with the Student's *t*-test for independent samples, assuming unequal variances. Relative risks were estimated as relative hazards, using the Cox proportional hazards model (42). The fit of the proportional hazards models was examined by analyzing changes in the proportionality of hazards with time and with risk factor levels. Risk factor-adjusted relative risks were estimated as antilogarithms of coefficients for independent variables. Covariates were entered uncategorized. All tests of significance were two-sided.

Both definite AMIs, fatal or nonfatal, possible AMIs and prolonged chest pains were defined as events. Deaths from causes other than acute myocardial infarction were defined as losses.

RESULTS

A total of 153 men (5.7 percent) of the 2,682 participants of the baseline examination had donated blood at least once in the 24 months preceding the baseline examination. Of the 153 donors, only one (0.7 percent) experienced an AMI during the follow-up, whereas of the 2,529 non-blood donors, 316 men (12.5 percent) had an AMI ($p < 0.0001$ for difference between proportions based on Pearson chi-square statistics).

To explore differences between blood donors and non-blood donors, the distributions of baseline characteristics of donors and non-blood donors were compared (tables 1 and 2). All traditional risk factors differed between donors and non-blood donors. Compared with the non-blood donors, the blood donors were on average younger, various measures of coronary disease were less common among them, their apolipoprotein B concentrations were on average lower, they smoked less, and their systolic blood pressures were lower. All differences except familial coronary disease were statistically significant ($p < 0.05$) by the Student's *t*-tests. All traditional risk factors were also independently statistically significant ($p < 0.05$) for AMI in a multivariate Cox proportional hazards model.

Psychosocial differences between blood donors and non-blood donors were analyzed using a step-up lo-

TABLE 1. Percent distribution of prevalent diseases and family history of coronary heart disease (CHD) in regular blood donors and non-blood donors among 2,682 men from eastern Finland at baseline examination in 1984–1989

Risk factor	Blood donors ($n = 153$)	Non-blood donors ($n = 2,529$)	p value for difference*
Diabetes mellitus	1.3	5.8	<0.001
Prevalent clinical CHD	8.5	26.3	<0.001
Ischemia in exercise test	19.6	36.1	<0.001
Family history of CHD	47.1	49.0	0.643

* From Student's *t*-tests assuming unequal variances comparing blood donors and non-blood donors.

TABLE 2. Distribution of other biologic, behavioral, and psychological risk factors in regular blood donors and non-blood donors among 2,682 men from eastern Finland at baseline examination in 1984–1989

Risk factor	Blood donors (n = 153)				Non-blood donors (n = 2,529)				p value for difference*
	Mean	SD†	Minimum	Maximum	Mean	SD	Minimum	Maximum	
Other biologic risk factors									
Age (years)	50.5	5.6	42.0	61.0	53.2	5.1	42.0	61.3	<0.001
Serum apolipoprotein B (g/liter)	0.97	0.25	0.19	1.72	1.04	0.24	0.19	2.38	0.001
Serum HDL_2† cholesterol (mmol/liter)	0.92	0.30	0.27	2.30	0.85	0.28	0.07	2.77	0.006
Systolic blood pressure (mmHg)	130.2	13.7	99	167	134.4	17.2	88	221	<0.001
Maximal oxygen uptake (ml/kg/minute)	35.3	6.8	18.5	56.5	30.1	7.6	6.4	65.4	<0.001
Behavioral risk factors									
Cigarette pack-years	4.1	10.0	0	50	8.7	17.2	0	174	<0.001
Dietary arsenic intake (mg/d)	45.6	27.9	16	253	47.6	33.5	9	651	0.397
Psychological risk factors									
Depression (MMPI† scored)	10.2	8.4	0	38	12.4	9.3	0	49	0.002
MMPI admission (MMPI scored)	10.0	6.0	0	31	12.3	6.8	0	36	<0.001

* From Student's t-tests assuming unequal variances comparing blood donors and non-blood donors.
† SD, standard deviation; HDL_2, high density lipoprotein subfraction 2; MMPI, Minnesota Multiphasic Personality Inventory.

gistic regression analysis. Of a total of over 79 psychosocial measurements including indices of depression, hopelessness, social networks and social support, cynical hostility, job demands, income, education, socioeconomic status, alexithymia and perceived health, Minnesota Multiphasic Personality Inventory depression, and admission scales differed most between donors and non-blood donors. Both of these indices were also associated with the risk of AMI. Blood donors and non-blood donors did not differ with regard to hopelessness, cynical hostility, and social isolation, characteristics that we have found previously to be predictive of coronary disease events.

In a Cox proportional hazards model adjusting for age and examination years, blood donors had a 94 percent lower risk of AMI (relative hazard = 0.058, 95 percent CI 0.008–0.414, p = 0.005) than non-blood donors. In a multivariate Cox model adjusting for all measured predictive coronary disease risk factors, blood donors had a 88 percent reduced risk (relative hazard = 0.120, 95 percent CI 0.017–0.857, p = 0.035) of AMI compared with non-blood donors (table 3).

In order to estimate the confounding effects of different categories of risk factors, we entered covariates concerning prevalent diseases and other biologic, behavioral, and psychosocial risk factors as four separate

TABLE 3. Relative hazards and 95% confidence intervals (CI) for acute myocardial infarction associated with regular blood donations and other strongest risk factors among 2,682 men from eastern Finland followed for an average of 9 years from baseline examination in 1984–1989*

Risk factor	Relative hazard	95% CI	p value
Blood donation	0.120	0.017–0.857	0.035
Prevalent diseases and family history			
Diabetes mellitus (yes vs. no)	1.97	1.41–2.75	<0.001
History of or prevalent clinical CHD† (yes vs. no)	1.84	1.35–2.50	<0.001
Family history of CHD (yes vs. no)	1.63	1.30–2.06	<0.001
Ischemia in maximal exercise test (yes vs. no)	1.29	0.95–1.77	0.107
Other biologic risk factors			
Serum apolipoprotein B (g/liter)	1.98	1.27–3.08	0.003
Serum HDL_2† cholesterol (mmol/liter)	0.51	0.31–0.84	0.007
Systolic blood pressure (10 mmHg)	1.09	1.03–1.16	0.006
Maximum oxygen uptake (ml/kg/minute)	0.957	0.939–0.976	<0.001
Behavioral risk factors			
Cigarette pack-years	1.009	1.003–1.015	0.004
Dietary arsenic intake (mg/d)	1.003	1.001–1.006	0.018
Psychological risk factors			
Depression (MMPI† scored)	1.018	1.004–1.033	0.015
MMPI admission (MMPI scored)	0.973	0.951–0.997	0.024

* The model also includes examination years (5 dummy variables), age (years), plasma fibrinogen (g/liter), serum selenium (μg/liter), plasma vitamin C (≤2.0 mg/liter vs. >2.0 mg/liter), hair mercury (≥2.0 ppm vs. <2.0 ppm), dietary iodine intake (μg/d), conditioning leisure-time physical activity (kcal/d), frequency of hangovers (times/year), annual gross income of the participant (1,000 FIM†), and place of residence (urban vs. rural) as covariates.
† CHD, coronary heart disease; HDL_2, high density lipoprotein subfraction 2; MMPI, Minnesota Multiphasic Personality Inventory; FIM, Finnish marks.

groups (table 4). The adjustment for prevalent cardiovascular diseases increased the relative hazard by 69 percent. Additionally, all other groups attenuated the association of blood donation with AMI risk, but less so.

To ensure that no other confounder would have explained the association of blood donations with AMI risk, we also entered in the model shown in table 3 all variables measured that had any association with the risk of AMI, one by one as covariates. The addition of any other variable measured (including diseases, other biologic factors, nutrients, other behavioral factors, and psychosocial factors) at the KIHD baseline examination had no notable effect on the relative hazard for blood donations. Blood donations had a significant association with the risk of AMI in all Cox models fitted.

DISCUSSION

The present study confirms our preliminary finding of an association between blood donations and a reduced risk of acute myocardial infarction (27). The association is strong and statistically significant in spite of the small number of blood donors in the study cohort. The association weakened but remained significant after statistical adjustment for the major risk factors for coronary disease as well as all other risk factors that were predictive in our study cohort. Because blood donors were generally more health-conscious and more healthy than those who had not donated blood, we carried out thorough data analyses in order to estimate the magnitude of this self-selection bias. A part of the observed association was explained by the confounding, but the residual association was still strong. On the basis of our data, we do not believe that confounding could have introduced the observed association.

Existing epidemiologic data on iron and coronary disease are inconsistent. In our previous study in 1,931 coronary disease-free men, high levels of stored body iron, assessed as serum ferritin ≥ 200 μg/liter, were associated with elevated risk of AMI, the risk being synergistically higher when combined with elevated low density lipoprotein cholesterol (8, 9). We have now repeated this study by using the ratio of transferrin receptors to ferritin as the measure of body iron stores (43). In a Canadian prospective population study in 9,920 men and women (21), elevated serum iron, both independently and combined with serum cholesterol concentration, was associated with an increased risk of fatal AMI (21). In the US Health Professionals Study in 44,933 men, high dietary intake of heme iron was associated with an increased risk of myocardial infarction (45). There are, however, also epidemiologic and other studies that failed to observe an association between iron and ischemic cardiac events. In these studies, large measurement variability

TABLE 4. Relative hazards and 95% confidence intervals (CI) for acute myocardial infarction associated with regular blood donations in different Cox models with different sets of covariates among 2,682 men from eastern Finland followed for an average of 9 years from baseline examination in 1984–1989*

Adjusted risk factors	Relative hazard	95% CI	p value
Age	0.058	0.008–0.414	0.005
Age + prevalent diseases and family history: maximal oxygen uptake, diabetes mellitus, prevalent clinical CHD†, CHD family history, exercise ischemia	0.098	0.014–0.700	0.021
Age + other biologic risk factors: systolic blood pressure, serum apolipoprotein B, HDL_2† cholesterol and selenium, plasma fibrinogen and vitamin C, and hair mercury	0.078	0.011–0.554	0.011
Age + behavioral risk factors: cigarette pack-years, conditioning LTPA†, hangover frequency, and dietary iodine and arsenic.	0.067	0.009–0.474	0.007
Age + psychosocial risk factors: depression, MMPI† admission, personal income, and place of residence	0.066	0.009–0.472	0.007
Age + all risk factors	0.120	0.017–0.857	0.035

* The model also includes examination years (5 dummy variables) as a covariate.
† CHD, coronary heart disease; HDL_2, high density lipoprotein subfraction 2; LTPA, leisure-time physical activity; MMPI, Minnesota Multiphasic Personality Inventory.

and use of different measures of iron stores are problems that may explain the lack of association, as discussed previously in detail (10, 11, 18–20).

Meyers et al. (28) published recently their study in which they observed a decreased incidence of cardiovascular events among 655 regular blood donors, as compared with 3,200 non-blood donors. No comprehensive measurements of potential confounders were, however, available to enable the study of the extent of confounding. Another problem in their study is that the outcome events were not validated from medical records.

Hematocrit is not a good indicator of body iron stores, because it is influenced by genetic factors, hormones, obesity, smoking and other factors that elevate active testosterone levels and enhance hematopoiesis. A number of prospective studies have observed an association between an elevated blood hematocrit and an increased risk of coronary and other cardiovascular events (8, 45, 46). Smoking, obesity, and testosterone activity are confounders of this association. The association has persisted, however, even after an adjustment for these risk factors (8, 45, 46). The effect of a blood donation on hematocrit lasts only for days to a few weeks. For this reason, the reduction of hematocrit is not likely to be the main mechanism through which blood donations could reduce the risk of AMI.

Harmfulness of high iron, or benefits of low iron, for the risk of coronary disease and/or for atherosclerotic progression does have some experimental, clinical, and epidemiologic support. Iron is a potent catalyst for free radical production in vitro, and iron exists both in the circulation and in tissue in forms capable to participate in radical producing reactions (5–7). In animal experiments, iron loading has promoted the progression of atherosclerosis in cholesterol-fed rabbits (14, 15), and in humans, pro-oxidative iron has been detected in fresh atherosclerotic material (16, 17). We have earlier demonstrated that repeated blood donations, in parallel with a decline in serum and erythrocyte ferritin, reduce the oxidation susceptibility of serum lipoproteins in men with elevated serum ferritin levels (47).

As a consequence of red blood cell loss due to blood donations, the production of new erythrocytes is accelerated to replace the lost ones. This consumes iron stores and leads to a reduction in serum ferritin concentration. This also means that the circulating red blood cells are on the average younger after a blood donation. It is possible that older erythrocytes leak both ferritin-bound and other chelated iron more than younger red blood cells. Thus, one can speculate that the effect of blood donations on the risk of AMI could be, besides through the decrease of body iron stores, also through the reduction of leakage of iron from red blood cells. Hemoglobin has been shown to promote lipid peroxidation in a number of in vitro studies (48, 49). We have also observed an association between elevated blood hemoglobin levels and increased ratio of antibodies against oxidized LDL to those against native LDL in a sample of 60 men, suggesting that hemoglobin might also induce lipid peroxidation in vivo (50).

Our present data suggest that blood donations may be associated with a decreased risk of AMI at least in the eastern Finnish male population. The impact of body iron on the incidence of AMI may be greater in this population than in many others because of the relatively high mean serum total cholesterol levels, assuming that the effect of iron and LDL cholesterol is synergistic (8, 10, 11, 21).

The association between donating blood and reduced risk of acute myocardial infarction needs to be confirmed in other prospective population studies. Furthermore, trials of the impact of iron depletion on atherosclerotic progression or coronary events are eventually necessary to verify or refute the theory concerning the role of excess iron and iron depletion in atherogenesis and in ischemic heart disease.

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