CME

Iron and cardiac ischemia: a natural, quasi-random experiment comparing eligible with disqualified blood donors

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BACKGROUND: The theory that elevated iron stores can induce vascular injury and ischemia remains controversial. We conducted a cohort study of the effect of blood donation on the risk of coronary heart disease (CHD) by taking advantage of the quasi-random exclusion of donors who obtained a falsely reactive test for a transmissible disease (TD) marker.

STUDY DESIGN AND METHODS: Whole blood donors who were permanently disqualified because of a false-reactive test between 1990 and 2007 in the province of Quebec were compared to donors who remained eligible, matched for baseline characteristics. The incidence of CHD after entry into the study was determined through hospitalization and death records. We compared eligible and disqualified donors using an "intention-to-treat" framework.

RESULTS: Overall, 12,357 donors who were permanently disqualified were followed for 124,123 person-years of observation, plus 50,889 donors who remained eligible (516,823 person-years). On average, donors who remained eligible made 0.36 donation/year during follow-up and had an incidence of hospitalizations or deaths attributable to CHD of 3.60/1000 person-years, compared to 3.52 among permanently disqualified donors (rate ratio, 1.02; 95% confidence interval, 0.92-1.13).

CONCLUSION: Donors who remained eligible did not have a lower risk of CHD, compared to donors who were permanently disqualified due to a false-reactive TD marker. Because of the quasi-random nature of false-reactive screening tests, this natural experiment has a level of validity approaching that of a randomized trial evaluating the effect of regular blood donation on CHD risk. These results do not support the iron hypothesis.

The so-called "iron hypothesis" suggests that iron plays a role in the pathogenesis of atherosclerosis and coronary heart disease (CHD).[1,2] This theory postulates that the oxidative properties of iron can induce chronic vascular injury, leading to the formation of atheroma and ischemia, and that decreased iron stores should therefore reduce the risk of CHD. Studies in animals and humans have produced conflicting results with regard to this theory.[3-9] The effect of iron stores on CHD has been studied in the context of voluntary blood donation, also with inconsistent results.[10-15] Concerns with these studies include the "healthy donor" bias, which if operative would show an apparently protective effect of blood donation on health outcomes because healthier people are more likely to donate, thus distorting the true association between donation and CHD.[16,17] The only randomized trial that addressed the iron hypothesis failed to show any beneficial effect of bloodletting among patients with established peripheral vascular disease.[18] However, this negative finding probably cannot adequately answer the question as to whether depleting the iron stores can prevent the occurrence of CHD in younger, healthier individuals, and it has been suggested that larger clinical trials in this population would be needed.[19]

We studied the effect of blood donation on the risk of CHD by taking advantage of the quasi-random exclusion

ABBREVIATIONS: CHD = coronary heart disease; HR = hazard ratio; RR(s) = rate ratio(s); TD = transmissible disease.

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of individuals who obtained a falsely reactive screening test for a transmissible disease (TD) marker as part of their donor qualification process. Over a period of more than 15 years, our transfusion center permanently disqualified several thousand otherwise healthy blood donors because of such false-reactive test results. This long-held practice resulted from regulatory constraints, despite the well-accepted notion that donors who falsely react on these tests are at no higher risk of infection compared to nonreactive donors.[20,21] In this cohort study, we retrospectively measured the incidence of CHD after the exclusion of potential donors because of false-reactive tests, which we compared to the incidence among donors who remained eligible and who were matched for certain baseline characteristics. CHD events were identified through administrative hospitalization and death records. Eligible and disqualified donors were compared according to an "intention-to-treat" framework, as would be done in a randomized trial.

MATERIALS AND METHODS

Study population

The study population comprised all donors who presented to make a whole blood donation in the province of Quebec between June 1990 and March 2007, with the exclusion of autologous donations. Héma-Québec (before 1998, the Red Cross) has the exclusive mandate for collecting blood in the province (population 8,000,000). Approximately 250,000 donations are made each year. All donations were systematically screened for a variety of TD markers and donations that were repeatedly reactive were evaluated with a confirmatory assay, for example a Western blot for human immunodeficiency virus (HIV). Donors who were repeatedly reactive on the screening assay were notified of their result and informed that they could no longer donate, and a permanent exclusion code was added to their record. The information on donors and their donations, including TD marker results and exclusion status, is maintained in a computerized system (PROGESA, Mak System, http://www.mak-system.net/). The start date of this cohort study corresponds to the time when we began the practice of permanently excluding donors with reactive TD screening tests, regardless of the confirmatory test results.

We included all donors who had reactive TD screening tests, but who obtained negative or indeterminate results on the confirmatory assay, for any of the following markers: antibodies against HIV, hepatitis C virus, human T-lymphotropic virus, syphilis, the hepatitis B surface antigen, and the p24 antigen. (The p24 antigen test was abandoned in 2003.) Donors with confirmed infection were excluded from this study. For each falsely reactive donor, we identified up to four donors who screened negative for all TD markers and therefore remained eligible to donate, whom we matched to those permanently disqualified for the following variables: age (within 10-year ranges), sex, first-time versus repeat donor, date of donation (±30 days), and region of residence.

CHD events (hospitalizations and deaths)

We used the name, surname, sex, date of birth, and residential address to trace donors in the Quebec universal health insurance registry, which also contains a personal health insurance number that allows tracking of patients in the health care system. All hospital admissions occurring in the province are recorded in the hospital discharge database (MED-ÉCHO), with the following information: date of admission, primary and secondary diagnoses, and medical and surgical interventions. All diagnoses are coded according to the International Classification of Diseases (ICD-9; ICD-10 since April 2006).[22] Interventions are coded using the Canadian Classification of Health Interventions (before April 2006, the Canadian Classification of Diagnostic, Therapeutic and Surgical Procedures).[23] Starting with the date of the falsely reactive TD marker or the corresponding date of donation in matched subjects, we identified all hospital admissions for which CHD was a primary or significant secondary diagnosis, or based on the occurrence of CHD-associated interventions, up to the end of the follow-up period in March 2009. The ICD-10 codes I20 to I25 and their corresponding codes in the ICD-9 system (410-414) were considered as CHD events. We also identified deaths occurring during the follow-up period, as recorded in the provincial death registry. This mandatory registry contains information on all deaths, including the date and the primary and up to 10 secondary causes of death, also coded according to ICD-9/10. We only considered deaths that were attributable to CHD.

Statistical analyses

Our analysis was planned to assess the hypothesis that the rate of CHD would be lower among donors who remained eligible, that is, potentially exposed to bloodletting, compared to disqualified (unexposed) donors. We assumed that any effect of iron reserves on atherosclerosis would likely take some time to become clinically manifest. Therefore, in the primary analysis, we required a 2-year delay after the date of inclusion in the study, before we started counting first occurrences only of CHD events (hospitalizations or deaths). Thus, person-years were accrued starting from the 2-year mark until the first event or until the end of the follow-up period. Only donors who could be traced in the health insurance registry were counted in both the numerator and the denominator data. Donors who died before the initial 2-year delay were considered lost to follow-up and observations on those who died of causes other than CHD were censored accordingly.

TABLE 1. Characteristics of potential study subjects, according to traceability within the provincial health care registry*

Baseline characteristics	Traceability in health care registry: Successful	Not successful	p value
Sex			
Female	25,551 (87.0)	3,809 (13.0)	<0.001†
Male	37,695 (95.8)	1,655 (4.2)	
Age (years)			
18-29	16,214 (95.1)	842 (4.9)	
30-39	17,772 (94.0)	1,142 (6.0)	
40-49	16,958 (90.3)	1,820 (9.7)	
50-59	9,737 (88.3)	1,284 (11.7)	
60+	2,565 (87.2)	376 (12.8)	
Mean ± SD	38.3 ± 12.1	42.7 ± 12.0	<0.001‡
Residence			
Montreal region	30,127 (90.5)	3,152 (9.5)	<0.001†
Quebec region	9,195 (94.5)	530 (5.5)	
Other	23,924 (93.1)	1,782 (6.9)	
Number of previous donations			
None	18,235 (92.0)	1,584 (8.0)	
1-3	26,522 (92.1)	2,289 (7.9)	
4-6	9,369 (91.4)	881 (8.6)	
7+	9,120 (92.8)	710 (7.2)	
Mean ± SD	3.3 ± 5.5	3.1 ± 5.1	0.76‡
Overall	63,246 (92.1)	5,464 (7.9)	

* Data are reported as number (%) unless otherwise specified.
† Chi-square test.
‡ Mann-Whitney U test.

Any CHD death occurring during hospitalization was considered to be the primary event. Rate ratios (RRs) and Kaplan-Meir estimates were calculated to compare the incidence of CHD between the two groups. In the main intention-to-treat analyses, eligible and disqualified donors were compared regardless of their actual donation behavior during the follow-up period. Cox regression was used to evaluate possible confounding of the association between exposure and CHD, in the event that baseline characteristics had become unbalanced after excluding untraceable subjects. Age, number of previous donations, and year of entry in the study were modeled as continuous variables; sex and region of residence were defined as categorical variables. The possible confounding effect of each baseline characteristic was individually and collectively assessed and the final models included all variables that meaningfully changed the unadjusted RRs. We also looked for possible modification of the effect of the exposure according to various categories of age and sex by including interaction terms in the multivariate models. All comparisons were made at the 95% confidence level (two-sided) using computer software (SAS EG, Version 4.1, SAS Institute, Cary, NC). Héma-Québec's ethical review board approved the study and the authorized government agency (Commission d'Accès à l'Information du Québec) gave permission to link databases.

RESULTS

We identified 13,753 donors who were permanently disqualified because of a falsely reactive screening test, and 54,957 matched donors who remained eligible to donate. Of these potential study subjects, 92.1% could be traced within the health insurance registry (92.6% among donors who remained eligible and 89.9% among those who were disqualified). As shown in Table 1, traceability was lower in women compared to men, in older compared to younger donors, and in those who live in the Montreal region compared to other regions. Traceability was higher for donors most recently entered into the study (data not shown, chi-square for trend, $p < 0.001$).

Table 2 shows that among donors who were successfully traced in the health insurance registry, the rate of hospitalizations or deaths due to CHD in those who remained eligible to donate was virtually the same as the rate among donors who were disqualified (3.60/1000 person-years and 3.52/1000 person-years, respectively), for a crude RR of 1.02 (95% confidence interval [CI], 0.92-1.13). Figure 1 shows the Kaplan-Meir plot of CHD incidence in both groups. Table 2 also shows measures of the association between baseline characteristics and the risk of CHD. As expected, increasing age and male sex were associated with higher rates of CHD, irrespective of donor status. There was a significant linear decrease in the incidence of CHD during the study period, with a RR of 0.97 (95% CI, 0.96-0.99) from year to year. The lack of association of CHD with exposure status (eligible compared to disqualified) remained unchanged after adjusting for any or all of the other baseline characteristics, including year at entry as a linear variable (adjusted hazard ratio [HR], 1.03; 95% CI, 0.93-1.15). When we examined the association separately among donors who were at least 40 years old at entry into the study and who thus had a higher probability of cardiac ischemia during follow-up, the risk of CHD still did not differ between eligible and disqualified donors (crude RR, 1.00; 95% CI, 0.89-1.12; adjusted RR, 1.04; 95% CI, 0.93-1.16). Findings were similar for donors less than 40 years old (crude RR, 1.00; 95% CI, 0.77-1.29; adjusted RR, 1.04; 95% CI, 0.80-1.34). The nonsignificant interaction term between eligibility status and age in this multivariate analysis ($p = 0.85$) confirmed the absence of any association between eligibility status and CHD in all age groups. The lack of an association between eligibility status and CHD was also consistent between men (adjusted RR, 1.06; 95% CI, 0.95-1.19) and women (adjusted RR, 0.75; 95% CI, 0.54-1.05), with a nonsignificant interaction term in the multivariate model ($p = 0.08$). The association between the number of donations before

TABLE 2. Hospitalizations or deaths attributable to CHD starting 2 years after entry in the study

	Eligible donors				Disqualified donors					
			Hospitalizations or deaths				Hospitalizations or deaths		Unadjusted	Adjusted
	Number (%)	Person-years	Number	Rate*	Number (%)	Person-years	Number	Rate*	RR (95% CI)	RR (95% CI)
Overall	50,889 (100)	516,823	1,727/133	3.60	12,357 (100)	124,123	410/27	3.52	1.02† (0.92 to 1.13)	1.03‡ (0.93 to 1.15)
Baseline characteristics										
Sex										
Female	20,732 (40.7)	205,813	158/10	0.82	4,819 (39.0)	46,667	42/2	0.94	Ref.	Ref.
Male	30,157 (59.3)	311,011	1,569/123	5.44	7,538 (61.0)	77,457	368/25	5.07	6.39§ (5.55-7.36)	4.61‖ (4.00-5.32)
Age (years)										
18-29	12,969 (25.5)	136,573	35/1	0.26	3,245 (26.3)	34,064	10/0	0.29	Ref.	Ref.
30-39	14,283 (28.1)	161,429	236/16	1.56	3,489 (28.2)	39,155	55/6	1.56	5.79§ (4.25-7.89)	5.10‖ (3.74-6.95)
40-49	13,714 (26.9)	134,690	630/46	5.02	3,244 (26.3)	31,194	143/7	4.81	18.5§ (13.7-24.9)	15.8‖ (11.7-21.3)
50-59	7,854 (15.4)	67,565	597/44	9.49	1,883 (15.2)	15,718	152/6	10.05	35.6§ (26.4-47.9)	29.8‖ (22.1-40.1)
60+	2,069 (4.0)	16,566	229/26	15.39	496 (4.0)	3,993	50/8	14.53	56.5§ (41.4-77.0)	42.7‖ (31.2-58.4)
Region										
Montreal	24,302 (47.8)	255,958	734/67	3.13	5,825 (47.1)	60,323	179/14	3.20	Ref.	Ref.
Quebec	7,373 (14.5)	72,075	271/17	4.00	1,822 (14.8)	17,909	63/2	3.62	1.25§ (1.11-1.41)	1.19‖ (1.06-1.35)
Other	19,214 (37.8)	188,791	722/49	4.08	4,710 (38.1)	45,891	168/11	3.90	1.29§ (1.18-1.41)	1.18‖ (1.08-1.29)
Previous donations										
None	14,611 (28.7)	146,108	340/21	2.47	3,624 (29.3)	35,959	81/2	2.31	Ref.	Ref.
1-3	22,373 (44.0)	232,475	710/53	3.28	4,149 (33.6)	44,999	148/12	3.56	1.36§ (1.22-1.53)	0.96‖ (0.85-1.07)
4-6	7,346 (14.4)	77,760	347/31	4.86	2,023 (16.4)	21,344	88/3	4.26	1.94§ (1.70-2.21)	1.00‖ (0.88-1.14)
7+	6,559 (12.9)	60,480	330/28	5.92	2,561 (20.7)	21,822	93/10	4.72	2.30§ (2.02-2.62)	0.97‖ (0.85-1.10)
Donations during follow-up										
None	17,132 (33.7)	158,162	602/37	4.04	12,321(99.7)	123,625	409/27	3.52		
1-3	18,025 (35.4)	182,793	603/54	3.59	31¶ (0.3)	426	1/0	2.35		
4-6	6,405 (12.6)	68,052	225/22	3.63	3¶ (0.0)	43	0/0	0.00		
7+	9,327 (18.3)	107,816	297/20	2.94	2¶ (0.0)	28	0/0	0.00		
Mean rate of donation	0.36/year				0.00/year					

* Per 1000 person-years, hospitalizations and deaths combined.
† Crude (unadjusted) RR comparing the rate of CHD of eligible and disqualified donors.
‡ RR comparing the rate of CHD of eligible and disqualified donors, adjusted for baseline characteristics.
§ Crude (unadjusted) RR, comparing the rate of CHD with the reference group of that baseline characteristic (including eligible and disqualified donors).
‖ Adjusted RR comparing the rate of CHD with the reference group of that baseline characteristic (including eligible and disqualified donors).
¶ A few donors were able to donate after being disqualified, due to constraints of the donor information system.

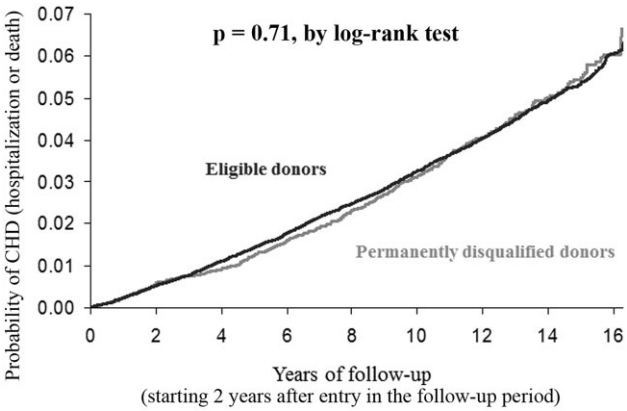

Fig. 1. Kaplan-Meier estimates of the probability of hospitalization or death caused by CHD according to study group.

entering the study and CHD disappeared after adjusting for age, as shown in the table. Table 2 also presents donation behavior in both groups of donors during the follow-up period. As expected, the rate of donation was virtually zero among disqualified donors. On average, donors who remained eligible donated at a rate of 0.36 donation/year and 33.7% never donated during follow-up.

When departing from the intention-to-treat framework and excluding from the analysis eligible donors who never returned to donate after entry into the follow-up period, the risk of CHD was still not significantly different when comparing eligible to disqualified donors (crude RR, 0.97, 95% CI, 0.87-1.08). We also calculated the incidence of CHD in the total follow-up period, without the initial 2-year delay; there was still no difference between eligible and disqualified donors (intention-to-treat analysis: crude RR, 1.04; 95% CI, 0.94-1.15).

In a secondary analysis, we restricted the study population to individuals who made at least four donations in the 2 years preceding the date of entry in the study (Table 3). This was done to increase the likelihood of more assiduous donation behavior among donors who remained eligible, which was indeed the case: 1.37 donations/year during follow-up and only 5.4% who never donated; the proportion who gave, on average, two or more donations each year during follow-up was 25.6%. In this subset of the overall study population, the incidence of CHD was still not different when comparing eligible with disqualified donors, both in the unadjusted and in the adjusted comparison, also with an intention-to-treat analysis (adjusted HR, 1.03; 95% CI, 0.78-1.37). The higher incidence of CHD in this subgroup of assiduous donors, compared to the total study population, is explained by their older age and a larger proportion of men at baseline, as shown in Table 3.

DISCUSSION

In this population-based study of individuals who self-selected for blood donation, our results do not support the iron hypothesis: donors who remained eligible did not experience a lower risk of CHD compared to donors who were disqualified because of a falsely positive TD screening test. A key strength of this study is that it can be seen as conceptually equivalent to a randomized experiment, assuming that false reactivity to a TD marker represents a chance event, unrelated to the risk of CHD.

We think that it is reasonable to assume that false reactivity is a chance event. False-reactive tests result from an idiosyncratic reaction between the test itself and the donor being tested.[24,25] This is supported by the observation that whenever a testing platform is replaced by a different technology, some habitual donors suddenly test false reactive.[26,27] There is no indication that a false-reactive test is related to an occult infection by the agent being targeted by the test. In fact, current regulations allow blood centers to reinstate falsely reactive donors, under certain conditions.[28] Finally, there is no reason to suspect that false reactivity is somehow associated with risk factors for CHD, such as smoking, diabetes, and lipid profile. If this had been the case, the resulting bias should have produced a difference in the rates of CHD between eligible and disqualified donors. Falsely reactive tests do not happen completely at random; they are more likely to happen in first-time donors, hence younger donors.[29] After properly matching for these and other characteristics, a comparison of eligible donors with falsely reactive, permanently disqualified donors represents a natural experiment that can be used to study the effect of donation on CHD.

Another important feature of this study is that it minimizes the healthy donor bias by including only prospective donors, who all self-selected to donate and who underwent the same detailed screening process to which donors are routinely subjected, including a health risk assessment, thus ensuring a homogeneous group of individuals. The study is also notable for its large sample size, resulting in rate estimates that have a high degree of precision.

We are confident that the vast majority of significant CHD events in our study subjects were captured through the Quebec administrative databases. These databases have previously been shown to provide accurate information on health outcomes, including cardiac diseases.[30-32] The high proportion of subjects who were successfully traced in the health insurance registry can be explained by the low mobility of Quebec residents. It is therefore unlikely that we missed a significant proportion of CHD outcomes. Population-based studies of CHD usually rely on hospitalization and death records, without including outpatient consultations.[33] We are confident that hospital-

TABLE 3. Analysis restricted to donors who had given four or more donations during the 2 years before their entry into the study

	Eligible donors				Disqualified donors					
			Hospitalizations/ deaths				Hospitalizations/ deaths			
	Number (%)	Person-years	Number	Rate*	Number (%)	Person-years	Number	Rate*	Unadjusted RR (95% CI)	Adjusted RR (95% CI)
Overall	3,571 (100)	34,523	186/21	6.00	1,213 (100)	11,604	57/6	5.43	1.10† (0.83 to 1.46)	1.03‡ (0.78 to 1.37)
Baseline characteristics										
Sex										
Female	896 (25.1)	8,711	7/1	0.92	269 (22.2)	2,441	5/0	2.05	Ref.	Ref.
Male	2,675 (74.9)	25,812	179/20	7.71	944 (77.8)	9,163	52/6	6.33	6.30§ (3.61-11.0)	4.67‖ (2.67-8.17)
Age (years)										
18-29	383 (10.7)	4,076	2/0	0.49	152 (12.5)	1,624	1/0	0.62	Ref.	Ref.
30-39	838 (23.5)	9,684	24/1	2.58	292 (24.1)	3,257	4/1	1.53	4.40§ (1.34-14.43)	3.79‖ (1.15-12.43)
40-49	1,108 (31.0)	10,897	51/7	5.32	379 (31.2)	3,757	19/1	5.32	10.1§ (3.19-32.0)	8.48‖ (2.67-27.0)
50-59	824 (23.1)	6,586	68/3	10.78	268 (22.1)	2,158	20/3	10.66	20.4§ (6.47-64.5)	18.3‖ (5.76-57.9)
60+	418 (11.7)	3,280	41/10	15.55	122 (10.1)	808	13/1	17.33	30.2§ (9.50-96.1)	26.6‖ (8.31-85.0)
Region										
Montreal	1,689 (47.3)	17,501	94/14	6.17	552 (45.5)	5,647	24/3	4.78	Ref.	Ref.
Quebec	643 (18.0)	6,165	35/2	6.00	212 (17.5)	2,049	14/0	6.83	1.06§ (0.77-1.47)	1.11‖ (0.81-1.54)
Other	1,239 (34.7)	10,857	57/5	5.71	449 (37.0)	3,908	19/3	5.63	0.98§ (0.74-1.28)	0.93‖ (0.70-1.22)
Previous donations										
4-6	755 (21.1)	7,647	28/3	4.05	168 (13.8)	1,864	11/0	5.90	Ref.	Ref.
7+	2,816 (78.9)	26,876	158/18	6.55	1,045 (86.2)	9,741	46/6	5.34	1.41§ (1.01-1.96)	1.00‖ (0.71-1.40)
Donations during follow-up										
None	194 (5.4)	1,603	19/4	14.35	1,209 (99.7)	11,545	57/6	5.46		
1-3	572 (16.0)	4,856	33/3	7.41	3¶ (0.2)	45	0/0	0.00		
4-6	562 (15.7)	4,809	35/4	8.11	0 (0.0)	0	0/0			
7+	2,243 (62.8)	23,255	99/10	4.69	1¶ (0.1)	15	0/0	0.00		
Mean rate of donation	1.37/year				0.00/year					

* Per 1000 person-years, hospitalizations and deaths combined.
† Crude (unadjusted) RR comparing the rate of CHD of eligible and disqualified donors.
‡ RR comparing the rate of CHD of eligible and disqualified donors, adjusted for baseline characteristics.
§ Crude (unadjusted) RR, comparing the rate of CHD with the reference group of that baseline characteristic (including eligible and disqualified donors).
‖ Adjusted RR comparing the rate of CHD with the reference group of that baseline characteristic (including eligible and disqualified donors).
¶ A few donors were able to donate after being disqualified, due to constraints of the donor information system.

ization and death records provide a robust and valid measurement of clinically significant ischemic heart disease, which most often leads to one of these outcomes. The addition of outpatient CHD diagnoses would have to rely on other administrative databases (e.g., physicians and drugs claims), which have significant limitations such as their high frequency of missing diagnoses.

The rates of CHD observed in our donors is similar to those reported for other donor populations. For example, the rate of CHD in the Health Professionals Follow-up Study was 3.2 per 1000 person-years among blood donors.[13] These rates are lower compared to the general population in Quebec and other industrialized countries, which likely reflects differences in donor demographics and underlying health status.[34,35] Finally, we believe that these results are representative of our total donor population, given that those few donors who could not be traced in the health care system differed only slightly on their baseline characteristics.

One limitation of the study is that a large proportion of eligible donors actually never donated during the follow-up period. The overall rate of donation was therefore modest in that group. It is conceivable that this low level of donation, which is analogous to noncompliance to treatment in the context of a randomized trial, could have masked a potential beneficial effect of lower iron reserves on CHD. However, we believe that our negative findings are nonetheless important. First, given the large sample size and the long duration of follow-up, we would have been able to detect a very small effect on the risk of CHD. Second, even when we departed from the intention-to-treat framework by excluding from the analysis eligible donors who never returned to donate during follow-up, there was still no association between the exposure and CHD. An even more compelling finding comes from our secondary analysis, in which we restricted the study population to donors who gave blood more frequently before entering the study. In that analysis (also done according to an intention-to-treat framework), we again found no difference in the rates of CHD comparing eligible to disqualified donors, in spite of the fact that donors who remained eligible continued to donate at a rate of 1.37 donations per year, a level of donation that would be expected to lower iron stores significantly. Our data cannot answer the question of whether a protective effect of donation can only occur among donors who have much higher rates of donation. However, if that is the case, the level of bloodletting that would provide some protection is likely to be such that only a minority of regular blood donors would benefit from this effect. It could also be argued that falsely reactive donors who previously donated had already benefitted from the alleged protective effect of iron store depletion on the risk of CHD. As shown in Table 2, approximately 70% of donors who had a false-reactive donation did make at least one prior donation. However, only a small proportion of donors with false-reactive tests had a high number of previous donations (seven or more, 20.7%). Furthermore, once they were permanently disqualified, these habitual donors no longer benefitted from this chronic exposure and their iron stores would eventually return to their baseline level. The differential exposure to iron depletion between disqualified and eligible donors was also enhanced by the 2-year delay that we allowed before counting CHD events in the follow-up period.

Another possible limitation of our study is that a proportion of potential study subjects could not be traced in the health care registry, with slight differences in baseline characteristics between traceable and untraceable subjects. Some differences have plausible explanations, for example, the fact that some women changed from maiden to married name or the greater mobility of donors who live in the Montreal area. These changes were not captured in the donor database if they happened after the last recorded donation, a situation that is more likely to happen among disqualified donors. These differences could suggest that the results cannot be readily generalized to the whole study population. However, the great majority of potential study subjects were successfully traced. Also, the differences between traceable and untraceable subjects, although significant due to the very large sample size, were small. Therefore, we are confident that the main results observed in traceable subjects, that is, the absence of an effect of donation on CHD risk, can be safely extrapolated to our entire donor population. Another concern could be that these slight differences in traceability could have introduced an imbalance in baseline characteristics between disqualified and eligible donors, since matching was done before determining traceability. However, as shown in Table 2, the distribution of these baseline characteristics was nearly identical between the two groups of donors who remained in the analysis. Furthermore, adjusting for these small differences in the multivariate analyses did not change the main finding of a lack of an association between eligibility status and CHD.

Our interpretation as to the absence of a protective effect of donation on CHD is strongly supported by the fact that the study was the result of a natural experiment, in which the appropriate comparison group is composed of people who had the same prior intent to donate; these people randomly lost the opportunity to give blood; therefore, self-selection and healthy donor bias should not have played any great role. The large health professionals follow-up study comparing donors to nondonors also reported no difference in the incidence of CHD.[13] As argued by the authors, that study was less prone to the healthy donor bias because it was conducted in a homogeneous, healthier group of health care workers. As suggested by others,[13,36] we suspect that the healthy donor

bias played a role in previous observational studies showing a lower incidence of CHD among regular donors compared to nondonors.[10-12]

Although a more recent study—which claimed to have avoided the healthy donor effect by comparing more frequent donors to occasional donors—reported a lower risk of CHD in more frequent donors, we think that this observation might be explained by donation frequency also being an indicator of underlying health.[14,16] Indeed, if we adopt a similar approach and restrict our analyses to donors who remained eligible, our own data can be analyzed to show that the incidence of CHD was lower in those who gave more frequently during the follow-up period (donors of four donations or more, compared to donors of zero to three donations: age and sex adjusted HR, 0.61; 95% CI, 0.55-0.67). This finding, instead of being explained by a protective effect of more frequent donations on the risk of CHD, is much more likely to be the result of reverse causation: donors who donated at a higher rate during follow-up did so because of their better health, rather than donation causing their healthier status. Even if a protective effect on CHD only happened at higher donation rates, this effect should have been detected when comparing all donors who remained eligible with those who were disqualified. The overall effect should have been even stronger in the secondary analysis of more assiduous donors, which included a substantial proportion of eligible donors who gave blood on a regular basis (two or more donations each year, 25.6%); our data do not even suggest such a trend.

Ideally, a large, randomized trial to study the effect of bloodletting on the risk of CHD in healthy volunteers would best address the iron hypothesis. Because of its design, we believe that the validity of our study comes very close to that of a true randomized experiment. Taken together, our findings and those of others cast serious doubts on the theory that reduced iron stores can protect against CHD, at least in the context of regular blood donation.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest relevant to the manuscript submitted to **TRANSFUSION**.

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