Blood donation and blood donor mortality after adjustment for a healthy donor effect

Henrik Ullum,[1] Klaus Rostgaard,[2] Mads Kamper-Jørgensen,[2,3] Marie Reilly,[4] Mads Melbye,[2] Olof Nyrén,[4] Rut Norda,[5] Gustaf Edgren,[4,6] and Henrik Hjalgrim[2]

BACKGROUND: Studies have repeatedly demonstrated that blood donors experience lower mortality than the general population. While this may suggest a beneficial effect of blood donation, it may also reflect the selection of healthy persons into the donor population. To overcome this bias, we investigated the relation between blood donation frequency and mortality within a large cohort of blood donors. In addition, our analyses also took into consideration the effects of presumed health differences linked to donation behavior.

STUDY DESIGN AND METHODS: Using the Scandinavian Donation and Transfusion database (SCANDAT), we assessed the association between annual number of donations in 5-year windows and donor mortality by means of Poisson regression analysis. The analyses included adjustment for demographic characteristics and for an internal *healthy donor effect*, estimated among elderly donors exempted from continued donation because of age criteria.

RESULTS: Statistical analyses included 1,182,495 donors of whom 15,401 died during 9,526,627 person-years of follow-up. Analyses adjusted only for demographic characteristics showed a 18.6% reduction in mortality per additional annual donation (95% confidence interval [CI], 16.8%-20.4%). After additional adjustment for the *internal healthy donor effect,* each additional annual donation was associated with a 7.5% decreased mortality risk 7.5% (95% CI, 5.7%-9.4%).

CONCLUSION: We observed an inverse relationship between donation frequency and mortality. The magnitude of the association was reduced after adjustment for an estimate of self-selection in the donor population. Our observations indicate that repeated blood donation is not associated with premature death, but cannot be interpreted as conclusive evidence of a beneficial health effect.

The number of whole blood donations from healthy nonremunerated blood donors varies between 16 and 60 per 1000 inhabitants per year in the United States and European Union.[1] Thus, possible health effects of blood donation, whether adverse or beneficial, are of public health importance. For instance, it is well established that repeated blood donations may result in iron depletion or deficiency,[2-5] which in turn may lead to anemia[3,4,6] and restless leg syndrome.[7] In recognition of these adverse effects of blood donation, iron supplementation is commonly encouraged to blood donors.

ABBREVIATIONS: MRR(s) = mortality rate ratio(s); SCANDAT = Scandinavian Donation and Transfusion database.

From the [1]Department of Clinical Immunology, Center of Clinical Investigation, Copenhagen University Hospital; the [2]Department of Epidemiology Research, Statens Serum Institut; and the [3]Department of Public Health, University of Copenhagen, Copenhagen, Denmark; the [4]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden; the [5]Department of Immunology and Transfusion Medicine, Uppsala University Hospital, Uppsala, Sweden; and the [6]Hematology Centre, Karolinska University Hospital, Stockholm, Sweden.

The creation of the SCANDAT database was funded by the National Heart, Lung, and Blood Institute; by the National Cancer Institute of the US National Institutes of Health (N01-CP-1175); and by the Danish Medical Research Council (271-08-0831/09-066021).

Address reprint requests to: Henrik Ullum, Department of Clinical Immunology 2034, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen East, Denmark; e-mail: henrik.ullum@regionh.dk.

Received for publication January 19, 2015; revision received April 16, 2015; and accepted April 20, 2015.

doi:10.1111/trf.13205

TRANSFUSION 2015;55;2479–2485

Epidemiologic studies have conversely demonstrated that compared with the general population, mortality,[8] cancer,[8,9] and cardiovascular disease[10-12] occurrence are markedly lower among blood donors. While this might reflect beneficial health effects of blood donation, it is an inherent challenge to the interpretation of such investigations that blood donors a priori represent a particularly healthy subgroup of the general population, active (i.e. current) blood donors presumably even more so than inactive (i.e., previous) donors. Thus, to be eligible both for initiation and for continuation of blood donation activity, individuals must meet certain health and lifestyle criteria. In addition, published surveys have demonstrated better self-reported health and healthier self-reported lifestyles in blood donors compared with the general population[13] (similar to unpublished results from the Danish Blood Donor Study[14]). This difference in self-perceived health is more marked for active than for inactive donors, suggesting that it is a strong determinant of blood donation activity.

In epidemiologic studies of donor health, the objective and self-perceived health criteria both contribute to selection bias, referred to as a *healthy donor effect*.[15,16] In practice, the healthy donor effect manifests as reverse causation; that is, it contributes to a lower disease occurrence in donors than in nondonors and in current donors than in previous donors, differences that could otherwise easily be misinterpreted as evidence of beneficial health effects of blood donation.[9,17]

In this investigation, we assessed the association between whole blood donation frequency and mortality employing two measures to account for the healthy donor effect. First, we nested our study within a large population-based cohort of blood donors thereby eliminating bias related to inclusion criteria. Second, we adjusted for any additional *internal* healthy donor effect relating to differences in donation intensity among the donors by considering mortality patterns in donors who ceased donation due to enforcement of age criteria.

MATERIALS AND METHODS

The Scandinavian Donation and Transfusion database

Our analyses were carried out within the Scandinavian Donation and Transfusion database (SCANDAT). The creation and update of the binational SCANDAT database has been described in detail elsewhere.[18,19] Briefly, in Sweden and Denmark blood banks belong to the public health care system. Since the late 1960s, an increasing number of blood banks in the two countries have employed computerized registration of their activities. SCANDAT contains all such electronic information on blood donors, donations, blood products, transfusions, and transfusion recipients available from blood banks in Denmark (1982-2012) and Sweden (1966-2012). Using the unique personal identification numbers issued to all citizens in the two countries as key, SCANDAT also contains information from nationwide health and population registers for donors and recipients, including vital status as of the end of 2012. This study was approved by appropriate scientific ethical committees and data protection agencies in both countries.

Calculation of individual donation rates

To ensure comparability between Denmark and Sweden, we disregarded donations before January 1, 1983, in this study. Analyses were restricted to allogeneic whole blood donations made by donors aged 18 to 64 years. In the individual blood banks, introduction of computerized registration did not entail retrospective recording of complete donation history of active donors. Consequently, donation history is incomplete (left truncated) for donors who were active at the time of initiation of computerized registration. We therefore focused exclusively on short-term effects of donation frequency. Specifically, at each time of donation, we ascertained the individual number of donations recorded during the preceding fixed-length window of 5 years and divided this number by 5. This donation intensity was used as the main exposure of interest in our study and was considered to remain constant until the next donation or end of follow-up, whichever came first. This assumption was made to avoid reverse causation when declining health or impending disease may result in cessation of donation. An example of the calculation of donation rates in 5-year exposure windows is illustrated in Fig. S1 (available as supporting information in the online version of this paper). In our analyses, the donation rates were arbitrarily classified into categories (0.01-0.50, 0.51-1.50, 1.51-2.50, 2.51-3.50, and 3.51-4.50 donations/year) with the 1.51 to 2.50 category including the median serving as a reference. Follow-up time with exposure levels greater than 4.5 donations per year was excluded, since only patients requiring therapeutic phlebotomy are bled more than four times per year in Sweden and Denmark. To test the robustness of our model to the choice of time windows, additional fixed-length exposure windows of 2 and 8 years were also considered.

Statistical analyses

Donors were followed from 5 years after the earliest recorded donation in SCANDAT until 5 years after the last recorded donation, the date of death, emigration, or end of 2012. For the supplementary analyses, follow-up was initiated 2 and 8 years after the earliest recorded donation and terminated 2 and 8 years after the last recorded donation, respectively.

The association between mortality and the donation rate in the most recent 5-year window was estimated by mortality rate ratios (MRRs) from Poisson regression

TABLE 1. Person-years, deaths, and all-cause MRR among blood donors in the moving 5-year observation window stratified according to age, sex, country, period, ABO blood type, D status, and Hb level*

Variable	Person-years (%)	Deaths (%)	MRR (95% CI)
Overall	9,526,627 (100)	15,401 (100)	
Age (years)			
18-39	4,002,391 (42.0)	2,012 (13.1)	0.28 (0.27-0.30)
40-59	4,577,519 (48.5)	8,068 (52.4)	1 (reference)
60+	946,717 (9.9)	5,321 (34.5)	3.25 (3.14-3.37)
Sex			
Male	5,328,601 (55.9)	11,228 (72.9)	1 (reference)
Female	4,198,026 (44.1)	4,173 (27.1)	0.59 (0.57-0.61)
Country			
Sweden	6,161,517 (64.7)	8,219 (53.4)	1 (reference)
Denmark	3,365,110 (35.3)	7,182 (46.6)	1.66 (1.61-1.72)
Period			
1983-1987	296,270 (3.1)	787 (5.1)	4.84 (4.46-5.26)
1988-1992	882,944 (9.3)	1,752 (11.4)	2.72 (2.56-2.89)
1993-1997	1,460,682 (15.3)	2,668 (17.3)	2.02 (1.92-2.13)
1998-2002	2,056,833 (21.6)	3,559 (23.1)	1.66 (1.58-1.75)
2003-2007	2,481,898 (26.1)	3,660 (23.8)	1.26 (1.20-1.32)
2008-2012	2,347,999 (24.6)	2,975 (19.3)	1.26 (1.20-1.32)
ABO blood type			
O	3,832,724 (40.2)	6,224 (40.4)	1 (reference)
A	4,132,489 (43.4)	6,651 (43.2)	1.01 (0.97-1.04)
AB	495,391 (5.2)	796 (5.2)	0.99 (0.94-1.05)
B	1,066,023 (11.2)	1,730 (11.2)	0.99 (0.94-1.05)
D status			
Positive	7,711,969 (81.0)	12,339 (80.1)	1 (reference)
Negative	1,814,658 (19.0)	3,062 (19.9)	1.04 (1.00-1.08)
Hb			
Low	895,724 (9.4)	1,861 (12.1)	1.48 (1.41-1.55)
Normal	8,270,384 (86.8)	12,565 (81.6)	1 (reference)
High	360,519 (3.8)	975 (6.3)	1.40 (1.31-1.50)

*MRRs are adjusted for sex, age, period, and country.

models adjusted for age (5-year intervals), sex, country, calendar period (5-year intervals), and hemoglobin (Hb) level categorized as low, normal, or high, with sex-specific limits (males, ≤134, 135-165, and ≥166 g/L; females, ≤120, 121-151, and ≥152 g/L). Donation frequency was treated as a time-dependent variable, and its effect on mortality was evaluated from trend tests describing the change in MRR with donation rate. In addition to the usual log-linear scale, we also investigated the exposure as a categorical variable with five levels. All statistical analyses were conducted with computer software (SAS, Version 9.1 or newer, SAS Institute, Cary, NC),[20] using the GENMOD procedure, after first stratifying and aggregating the follow-up time and deaths using the *stratify* macro.[21]

Adjustment for the healthy donor effect

We estimated potential internal healthy donor effects relating to differences in donation intensity between donors by focusing on donors who had ceased donating because of age criterion enforcement. Specifically, the maximum age for donation in both Sweden and Denmark was 65 years, and we therefore considered donors who had last donated at ages 64.5 to 65 years to meet this criterion. To reduce any short-term causal effects of donation per se even further, we considered only mortality among "retired" donors who survived at least 2 years after their most recent donation.

We defined donor follow-up time satisfying these two criteria as "retired" follow-up time and the habitual donation pattern of these retired donors was estimated as the donation rate in the relevant exposure window at the time they ceased donating. The full (adjusted) model for the association between donation pattern and death included variables for the donation rate, an indicator for ongoing donation (i.e., not yet retired) and the interaction between these two variables. In this model the variable representing the main effect of donation is identified among the "retired" donors (i.e., the healthy donor effect) and by adjusting for this in our model, the interaction between being non-"retired" and donation rate yields the variable of interest: the association between death and donation rate, adjusted for the healthy donor effect. For the supplementary analyses with 2-year exposure window "retired" donors were followed up for 5 years after the last donation.

RESULTS

We followed 1,182,495 donors for 9,526,627 person-years of follow-up during which time 15,401 deaths occurred. The distribution of person-years, deaths, and estimated MRRs are presented in Table 1 stratified according to age,

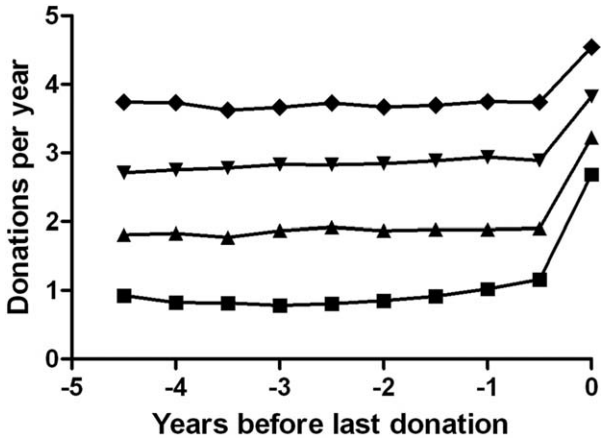

Fig. 1. Mean donation rates per person year calculated in half-year intervals plotted among donors who died at age less than 65 within 5 years after their last donation, donated more than two times in the 5-year window preceding their last donation, and started to donate more than 5 years before the latest donation. The time windows and the donation groups are defined at the latest donation recorded. (■) 0.51 to 1.50; (▲) 1.51 to 2.5; (▼) 2.51 to 3.50; (◆) 3.51 to 4.50.

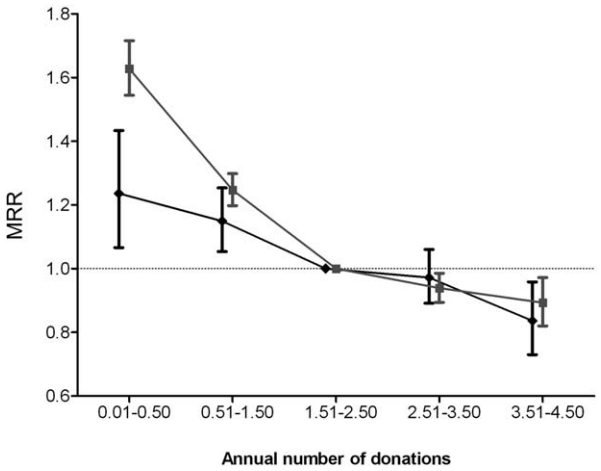

Fig. 2. MRR with and without adjustment for the healthy donor effect depicted according to the number of donations given per year during the stepping exposure windows of 5 years. Reference category was set to 1.51 to 2.50 donations per year (MRR, 1). All data are adjusted for age (in 5-year intervals), sex, country, and calendar period. Vertical bars represent 95% CIs. Results of trend tests are presented as p values. (◆) Healthy donor adjusted; MRR, 0.92 (0.91-0.94); $p < 0.0001$. (■) Healthy donor unadjusted; MRR, 0.81 (0.80-0.83); $p < 0.0001$.

sex, country, calendar period, ABO blood group, D status, and Hb level. Older age, male sex, Danish residence, and earlier calendar period were all associated with increased mortality. Table 1 further shows that both elevated and decreased Hb levels were associated with increased mortality. D– individuals had a 4% higher mortality risk ($p = 0.04$), whereas there was no association with ABO blood group. D– donors had a higher mean donation rate (1.6 per year) compared to D+ donors (1.5 per year).

To validate if donation activity gradually decreased shortly before serious disease (death), we depicted donation rates for donors who had died within 5 years after their last donation and before their 65-year birthday (Fig. 1). The data suggested that donors generally maintain a steady donation activity until they simply stop donating shortly before they fall ill. The increase in donation frequency closest to death suggested by Fig. 1 is a simple mathematical implication of the estimation of donation rate at the date of the most recent donation. We therefore concluded that calculation of donation intensity with our stepping time window was a meaningful measure of exposure.

We estimated an internal healthy donor effect related to donation frequency in follow-up analyses of donors who had donated after the age of 64.5 years (Fig. S2, available as supporting information in the online version of this paper). These donation rate–specific MRRs were included in Poisson regressions models of mortality among all donors.

Figure 2 shows MRR with and without adjustment for the internal healthy donor effect in addition to adjustment for age, sex, period, and country. The analysis not adjusted for the internal healthy donor effect demonstrated that each additional annual donation was associated with a 18.6% (95% confidence interval [CI], 16.8%-20.4%) reduction in mortality. In the analysis adjusted for internal healthy donor effect increasing donation frequency was still associated with decreased mortality, but to a lesser extent, with each additional annual donation now associated with a 7.5% (95% CI, 5.7%-9.4%) reduction in mortality. Similar patterns were observed in the 2- and 8-year windows (Figs. S3A and S3B, respectively; available as supporting information in the online version of this paper). Table 2 displays healthy donor-adjusted MRR after stratifications for sex, country, period, ABO blood group, D blood type, and Hb level. Stratified analyses generally showed similar results with the exception of donors with low Hb levels and with blood group AB and B.

DISCUSSION

In accordance with previous reports,[10,13,22] we observed lower all-cause short-term mortality among high-frequency donors compared to low-frequency donors. This variation in mortality could reflect a beneficial health effect of repeat blood donation or inherent health differences (i.e., an internal healthy donor effect) between high- and low-frequency donors or both. To investigate this, we estimated the internal healthy donor effect among active donors forced to retire from donating because of

TABLE 2. All-cause MRR among blood donors according to donation rate in 5-year observation windows stratified according to sex, country, period, ABO blood type, D status, and Hb level*

	Number of donations/year					
Variable	0.01-0.50	0.51-1.50	1.51-2.50	2.51-3.50	3.51-4.50	Per donation
Overall	1.24 (1.07-1.43)	1.15 (1.05-1.25)	1 (reference)	0.97 (0.89-1.06)	0.84 (0.73-0.96)	0.88 (0.85-0.91)
Sex						
Male	1.24 (0.05-1.46)	1.20 (1.09-1.32)	1 (reference)	1.02 (0.93-1.13)	0.86 (0.75-0.99)	0.89 (0.86-0.92)
Female	1.29 (0.89-1.87)	1.00 (0.83-1.20)	1 (reference)	0.80 (0.66-0.98)	0.87 (0.42-1.81)	0.84 (0.78-0.91)
Country						
Denmark	1.34 (1.12-1.60)	1.18 (0.05-1.34)	1 (reference)	1.00 (0.88-1.13)	0.85 (0.67-1.08)	0.87 (0.83-0.91)
Sweden	1.21 (0.92-1.60)	1.12 (0.99-1.27)	1 (reference)	0.95 (0.84-1.07)	0.83 (0.70-0.99)	0.88 (0.84-0.92)
Period						
1983-1992	1.44 (0.98-2.12)	1.25 (0.93-1.67)	1 (reference)	1.10 (0.67-1.80)	0.86 (0.45-1.64)	0.78 (0.70-0.87)
1993-2002	1.25 (1.02-1.54)	1.14 (0.99-1.30)	1 (reference)	0.88 (0.75-1.03)	0.70 (0.55-0.90)	0.82 (0.78-0.87)
2003-2012	0.91 (0.68-1.22)	1.09 (0.96-1.24)	1 (reference)	1.02 (0.91-1.13)	0.89 (0.75-1.06)	0.95 (0.91-1.00)
ABO blood group						
O	1.23 (0.99-1.54)	1.26 (1.09-1.45)	1 (reference)	1.02 (0.90-1.17)	0.87 (0.71-1.07)	0.87 (0.83-0.92)
A	1.34 (1.04-1.73)	1.13 (0.99-1.29)	1 (reference)	0.92 (0.80-1.04)	0.80 (0.65-0.99)	0.85 (0.81-0.90)
AB	0.97 (0.55-1.70)	0.80 (0.57-1.11)	1 (reference)	0.89 (0.55-1.41)	0.67 (0.34-1.31)	0.98 (0.85-1.13)
B	1.13 (0.77-1.67)	1.17 (0.91-1.49)	1 (reference)	1.06 (0.78-1.43)	0.91 (0.55-1.48)	0.95 (0.86-1.05)
D blood group						
D+	1.26 (1.07-1.49)	1.16 (1.06-1.28)	1 (reference)	0.98 (0.89-1.08)	0.83 (0.71-1.97)	0.88 (0.85-0.91)
D−	1.13 (0.81-1.56)	1.09 (0.89-1.33)	1 (reference)	0.94 (0.78-1.14)	0.85 (0.63-1.15)	0.86 (0.80-0.93)
Hb level						
Low	0.87 (0.58-1.32)	0.99 (0.76-1.29)	1 (reference)	1.18 (0.88-1.57)	0.77 (0.54-1.10)	0.98 (0.89-1.08)
Normal	1.21 (1.03-1.42)	1.15 (1.05-1.27)	1 (reference)	0.95 (0.86-1.04)	0.87 (0.74-1.01)	0.87 (0.84-0.91)
High	2.42 (1.16-5.03)	1.14 (0.98-2.02)	1 (reference)	1.07 (0.72-1.60)	0.68 (0.40-1.15)	0.82 (0.72-0.93)

* MRRs are adjusted for sex, age, period, country, and healthy donor effect. Per donation shows MRR per extra donation calculated with donation rate on a log-linear scale.

advanced age. Consistent with our expectations, inclusion of this variable in the statistical model attenuated the inverse association between donation frequency and all-cause mortality considerably, but did not explain it entirely. The remaining weaker inverse correlation might represent a true beneficial effect of donations per se, but could also reflect residual confounding given the crude nature of our estimation of the internal healthy donor effect. This interpretation of data is supported by a slightly higher mortality risk among D– donors despite the fact that they had a higher donation rate. Thus, while our data are reassuring in the sense that they do not suggest any overall deleterious effects of blood donation on blood donor health, they cannot be interpreted as conclusive evidence of a beneficial health effect either.

In a previous investigation exploring the association between blood donation and cancer, we defined donation frequency exposure with a lag period of 2 years to avoid reverse causality, that is, that impending but undiagnosed cancer would cause the donor to donate less frequently.[9] While plausible for a study of cancer outcomes,[9] where one may assume a lengthy induction period, the downside of this design was that we were unable to estimate immediate effects of donation frequency, that is, within 2 years of donation. In the present context, we could not assume that potential associations between blood donation and death would predominantly occur after a fixed lag period. We therefore measured donation rate in a time window of fixed length before the time of the most recent donation. If donors with impending life-threatening disease gradually ceased to donate, the donation rates calculated in this window would also be prone to reverse causality. However, we observed no such gradual decline in donation frequency before death. Therefore, the moving time windows we used for exposure assessment will be less susceptible to reverse causality and can be used without a lag period.

Our investigation has a number of strengths and weaknesses. We overcame selection bias by restricting our analyses to blood donors and including all registered donors in a binational database. Moreover, our analyses included adjustment for a presumed internal healthy donor effect linked to donation behavior. Despite the novelty of this approach and its advantage compared to previous studies, it is not without limitations. First, the estimation in the elderly donors may not capture the internal healthy donor effect entirely, as some older donors are in fact allowed to donate even past age 65 years. Second, we assumed that the healthy donor effect is uniformly distributed across age groups, which may not be the case. Finally it is important to note that our calculated MRRs are defined internally by comparing donors with different donation rates and that causality cannot be inferred by such observational data.

In conclusion, we demonstrate that even within the donor population, factors influencing donation behavior may also be predictive of mortality, thereby constituting an internal health donor effect. Our analyses reassuringly

showed that, if anything, increasing blood donation frequency was associated with a marginally decreased mortality. The inverse correlation between blood donation frequency and mortality was to a considerable degree explained by the internal healthy donor effect leaving little evidence that repeat donation has a true independent effect on donor mortality. The most pronounced physiologic effect of whole blood donation is a reduction of iron stores. If there was a true protective effect of iron depletion through blood donation we would expect the effect to be stronger among men since they have higher iron levels and are at lower risk of iron deficiency.[5] However, if anything the association between donation intensity and mortality is smaller in men than in women. Accordingly, we find it likely that previous reports of reduced mortality among blood donors[8] and of reduced morbidity among frequent blood donors[10,11] are mostly expressions of the healthy donor effect. However, considering the uncertainties in such observational data and the large number of blood donations globally, further studies are warranted.

ACKNOWLEDGMENTS

HU, MKJ, HH, KR, and GE planned the study; HH, GE, MR, KR, MM, and ON were responsible for the data collection; MKJ and KR were responsible for the data analysis; HU wrote the first draft of the paper; and all authors contributed to the final version.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Fig. S1. The figure depicts an example of the calculation of the mean number of donations per year in an

exposure window of 5 years. At each new donation the exposure is defined as the mean number of donations per year during the preceding 5 years. At time of the next donation, the exposure level is recalculated. For the donor in this example, follow-up starts in 1990, 5 years after the first donation in 1985. In the period from 1985 to 1990 the donor donates regularly (twice yearly) and in 1990 a rate of 2.0 donations per year has been reached with 10 donations in the preceding 5 years, and this rate is maintained for the next 5 years. From 1995 until the end of follow-up in 2001 only a single donation is recorded in mid-1998. At the time of this donation the exposure level is 1 donation per year as there were a total of 5 donations in the preceding 5 years. This exposure level is applied until end of follow up as no further donations occurred.

Fig. S2. Healthy donor effect expressed as mortality rate ratios (MRRs) among donors who gave their last donation at age 64.5-65 and who survived 2 years thereafter, stratified by average number of donations per year during the 5-year exposure window preceding the last donation. MRRs are adjusted for age (in 5-year intervals), sex, country, and calendar period. The reference category was set to 1.51-2.50 donations per year (MRR = 1). Vertical bars represent 95% confidence intervals.

Fig. S3. Mortality rate ratio with and without adjustment for the healthy donor effect is depicted according to the average number of donations per year during a moving exposure window of 2 years (Fig. A) and 8 years (Fig. B). Reference category was set to 1.51-2.50 donations per year (mortality rate ratio = 1). All data are adjusted for age (in 5-year intervals), sex, country, and calendar period. Vertical bars represent 95% confidence intervals. Results of trend tests are presented as p values.