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Higher usual alcohol consumption was associated with a lower 41-y mortality risk from coronary artery disease in men independent of genetic and common environmental factors: the prospective NHLBI Twin Study[1,2]

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ABSTRACT

Background: Evidence that alcohol consumption is inversely associated with long-term coronary artery disease (CAD) mortality independent of genetic and early life environmental factors is lacking.
Objective: We evaluated whether alcohol consumption was prospectively associated with CAD mortality risk independent of familial factors.
Design: In total, 843 male twins (396 pairs and 51 unpaired twins) aged 42–55 y (mean: 48 y) without baseline CAD reported beer, wine, and spirits consumption at baseline (1969–1973) and were followed up to 2010 in the prospective National Heart, Lung, and Blood Institute Twin Study. Data on usual alcohol consumption over the past year were collected. Outcome was time to event, where the primary event was death from CAD and secondary events were death from cardiovascular disease and all causes. HRs were estimated by using frailty survival models, both overall and within-pair.
Results: There were 129 CAD deaths and 219 cardiovascular deaths during 41 y of follow-up. In the whole cohort, after adjustment for caloric intake and cardiovascular disease risk factors, overall HRs per 10-g increment in alcohol intake were 0.94 (95% CI: 0.89, 0.98) for CAD and 0.97 (95% CI: 0.93, 1.00) for cardiovascular mortality. The within-pair adjusted HRs for a twin with 10-g higher daily alcohol consumption than his co-twin were 0.90 (95% CI: 0.84, 0.97) for CAD and 0.95 (95% CI: 0.90, 1.00) for cardiovascular disease mortality in the cohort pooled by zygosity, which remained similar among monozygotic twins. All 3 beverage types tended to be associated with lower CAD mortality risk within-pair to a similar degree. Alcohol consumption was not associated with total mortality risk overall or within-pair.
Conclusion: Higher usual alcohol consumption is associated with lower CAD mortality risk, independent of germline and early life environment and adulthood experience shared among twins, supporting a possible causal role of alcohol consumption in lowering CAD death risk. This trial was registered at clinicaltrials.gov as NCT00005124. *Am J Clin Nutr* 2015;102:31–9.

Keywords: alcohol, monozygotic twins, dizygotic twins, coronary artery disease, mortality

INTRODUCTION

Usual alcohol consumption is associated with a reduced risk of coronary artery disease (CAD)[8] (1–4). In the epidemiologic field, the consumption of alcohol over the previous 1 y is considered representative of usual intake (5). A number of potential confounding factors influence this association, not all of which are commonly included in long-term cohort analyses. For example, genetic factors, early life experience, and environmental factors all affect alcohol drinking behavior (6–8) and CAD mortality (9–11). Environmental factors related to alcohol drinking include alcohol supply, accessibility, and affordability; social and cultural norms or attitude toward alcohol drinking; and legal regulations of alcohol drinking (12–14). It is unclear whether alcohol consumption is inversely associated with long-term CAD mortality risk independent of genetic and early life environmental factors.

Although traditional observational studies generally do not control for genetic and shared environmental factors well, co-twin control studies represent a unique approach for holding these factors constant. Monozygotic co-twins share 100% of their germline (genes and inherited epigenetic modifications), and dizygotic co-twins share roughly 50% on average (15). A co-twin control design in which co-twins within a pair are compared with each other is uniquely suited to control for germline and early life

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[2] Supplemental Tables 1–5 are available from the "Supplemental data" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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[8] Abbreviations used: CAD, coronary artery disease; ICC, intraclass correlation coefficient; ICD-9, International Classification of Diseases, Ninth Revision; NHLBI, National Heart, Lung, and Blood Institute.

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and adulthood environment shared between co-twins, considering that they are naturally matched for the shared factors (15).

Only one twin study has attempted to examine the prospective association of alcohol consumption with total cardiovascular death but not CAD (16). This 24-y follow-up twin study found 2-fold higher total cardiovascular mortality among abstaining monozygotic twins compared with their light-drinking twin brothers, but the association was limited to nonsmokers in whom only 25 cardiovascular deaths occurred (16). A study based on the Swedish Twin Registry did not specifically use a co-twin control design to fully account for genetic factors and common or shared environment (17). As a result, it remains unclear whether the association between usual alcohol consumption and long-term mortality risk from CAD is independent of germline and shared early life environment and adulthood experience. The objective of our study, therefore, was to address this unresolved question by using a co-twin control design within a prospective cohort study with follow-up over several decades.

METHODS

Study population

As described previously (18, 19), the National Heart, Lung, and Blood Institute (NHLBI) Twin Study is a multicenter, population-based, prospective study on cardiovascular disease risk with emphasis on genetic and environmental factors in the United States. Initiated in 1969, the NHLBI Twin Study enrolled 514 middle-aged, white male veteran twin pairs [1028 men, 254 monozygotic and 260 dizygotic twin pairs (18)] from the National Academy of Sciences–National Research Council Veteran Twin Registry who lived within 200 miles of 5 research centers: Framingham, Massachusetts; San Francisco, California; Davis, California; Los Angeles, California; and Indianapolis, Indiana (15). These twins were born between 1917 and 1927 (18), a period when at most 2 male monozygotic twin pairs and 4 male dizygotic twin pairs were born per 1000 live births (20–22). All twins were physically examined at baseline and during follow-up studies by using the well-established Framingham Heart Study protocol to ensure the uniform examination of all twins by experienced cardiovascular epidemiologists (19). Zygosity was ascertained by 8 red blood cell antigen groups (serotyping 22 erythrocyte antigens) in the 1960s and variable number of tandem repeat DNA markers in the 1980s (18).

We excluded 59 twin pairs without baseline dietary data and 61 twins with baseline CAD. To account for potential effects of preexisting illness on the association between the baseline alcohol consumption and subsequent disease, an additional 7 twins were excluded due to deaths occurring within the first 2 y after the baseline investigation. The sample for analyses included 843 twins (205 monozygotic and 191 dizygotic twin pairs, 25 unpaired monozygotic and 26 unpaired dizygotic twins; an unpaired twin was a twin who was included in the analysis without his co-twin). These twins were followed up for 41 y with a total of 25,262 person-years of follow-up. Excluded twins were older, were less likely to be current smokers, were more likely to use antihypertensives, had higher blood concentrations of LDL cholesterol and postload glucose, and had a higher ratio of HDL cholesterol to triglyceride than the retained twins. The NHLBI Twin Study was approved by the institutional review board at each examination site, and all twins gave written informed consent. Our study was approved by the institutional review boards at Indiana University and Vanderbilt University.

Assessment of alcohol consumption

Through a standardized nutritionist-administered, cross-checked, dietary history interview adapted from the method of Burke (23) and validated in the Framingham study (24, 25), data on usual alcohol consumption and other dietary factors at baseline (examination 1) were collected. At examinations 2 and 3 (26), alcohol consumption data were again collected. At examination 1, one serving of an alcoholic beverage was defined as a 12-oz (355-mL) bottle of regular beer, a 3.5-oz (104-mL) glass of wine, or a 1.5-oz (44-mL) glass of spirits (27). The ethanol (pure alcohol) content per serving was roughly 14 g for beer, 10 g for table wine, or 17 g for spirits (27). At examinations 2 and 3, using the same serving size as for examination 1, we defined the ethanol content per serving as 14 g for beer, 10 g for table wine, and 16 g for spirits, based on their average ethanol content from the USDA Nutritive Value of Foods 1970 (28) and 1991 (29) revisions.

Assessment of covariates

Through in-person interview and physical examination, baseline data on all other major cardiovascular disease risk factors were collected (15). Data on age, years of education, marital status, and smoking status (current smoker, past smoker, and never smoker) were collected. Weight and height were measured. Systolic and diastolic blood pressures were measured by using a standard mercury sphygmomanometer (30). Triglycerides, total cholesterol, HDL cholesterol, and LDL cholesterol in plasma were measured after at least a 9-h overnight fast by using North American Lipid Research Clinics methodology (30). Plasma glucose concentrations at 1 h after a 50-g glucose load were measured among those without a previous diagnosis of diabetes. Baseline diabetes was defined by current use of insulin or oral hypoglycemic agents, or a 1-h post-50-g glucose load plasma glucose concentration $>$250 mg/dL, as described previously (30, 31). A 12-lead electrocardiogram was recorded. Information on current use of medications was collected. Participants were interviewed by a physician who completed a medical history questionnaire that included questions about cardiovascular events and procedures (32). Heart disease and other forms of cardiovascular disease were diagnosed by the physician at baseline (32).

Assessment of endpoints and follow-up

Vital status and the cause and date of death through 31 December 2010 were ascertained through medical records in follow-up examinations (examination 2, 1981–1982; examination 3, 1986–1987; examination 4, 1995–1997; and examination 5, 1999–2000), death certificates, or the National Death Index (32). As described previously (15, 32), criteria used for ascertaining outcomes in follow-up examinations were standardized, and decisions regarding disease diagnosis were made by a panel of investigators: at examinations 2 and 3, 2 independent physicians reviewed medical records; at examinations 4 and 5, one physician reviewed medical records. Physicians assigned corresponding International

Classification of Diseases, Ninth Revision (ICD-9) codes. Death certificates or the National Death Index coded to the ICD-9 were obtained for decedents. The primary endpoint was death from CAD (ICD-9 410–414) as the underlying death cause. Secondary endpoints were death from cardiovascular diseases (ICD-9 390–398, 402, 404, and 410–438); the underlying death cause, including CAD, heart failure, and hemorrhagic and ischemic stroke; and all causes. Subjects were considered lost to follow-up if a death certificate or coding from the National Death Index could not be traced. Nineteen of the 843 twins were lost to follow-up. These twins were included in this study and were treated as if they were alive at the date of the end of the study (15). The twins lost to follow-up were similar to other twins except that they were less likely to be currently married. The follow-up was terminated at the date of death, end of follow-up, or loss to follow-up, whichever occurred first.

Statistical analysis

We used a random coefficient model to estimate the age-adjusted intraclass correlation coefficient (ICC) for alcohol consumption by zygosity. The heritability was calculated by using Falconer's formula: $2 \times (ICC_{monozygotic} - ICC_{dizygotic})$, and common environmental contribution was calculated as $2 \times ICC_{dizygotic} - ICC_{monozygotic}$ (33). We compared demographic and clinical characteristics according to alcohol use by using linear mixed models for continuous variables, generalized estimating equation logistic models for dichotomous variables, and repeated proportional odds models with generalized estimating equations for categorical smoking and marital status variables.

We estimated HRs and 95% CIs by using the frailty survival model to account for natural clustering within a twin pair (15, 34) through a frailty (i.e., a random effect). We followed the modeling strategy for twin data analyses described previously (15, 35, 36). To be comparable to a general population study, we first evaluated the overall or individual association by treating twins as individuals accounting for within-pair clustering, where individual alcohol consumption (the overall effect) was an exposure variable. Next, we performed our primary analyses of the within-pair association to control for potential genetic and early life environmental confounding in a matched analysis for the co-twin control design. We used a within-pair effect and between-pair effect model (15, 35). The within-pair effect was the exposure variable. It was parameterized as the deviation of a twin's alcohol consumption from the mean consumption of the twin pair. The between-pair effect, the matched variable and predictor, was parameterized as the mean alcohol consumption between co-twins of a twin pair and represented effects from germline and environment shared between co-twins (15). Because monozygotic co-twins share 100% of genes while dizygotic co-twins, on average, share 50% of the segregating genes, any difference within a monozygotic twin pair should be caused by environmental factors (15). The within-pair association was independent of confounding from germline and shared environment (15, 35), regardless of whether an interaction existed between within-pair association and zygosity (15). Where that interaction was not statistically significant (i.e., the effects of alcohol were similar between monozygotic and dizygotic twin pairs), we pooled data for all twin pairs (37).

We modeled alcohol consumption in several ways. We primarily analyzed usual alcohol consumption as a simple continuous variable after confirming the lack of a nonlinear relation through tests using both a quadratic term ($P_{quadratic\ term} > 0.05$) and restricted cubic splines (38, 39) ($P > 0.05$). We also examined intake in quintiles for illustrative purposes. To avoid overfitting our models, particularly for analyses among monozygotic twins, we constructed a modified Framingham Risk Score following the published method (15, 40). This modified score was a composite score of 7 known cardiovascular disease risk factors: age, smoking, systolic and diastolic blood pressure, cholesterol in HDL and LDL, and diabetic status (40). A higher score was strongly associated with a worse 41-y mortality risk from CAD (HR per unit increment in the score, 1.08; 95% CI: 1.05, 1.11) in our cohort. We also tested the associations by use of individual covariates of this score in sensitivity analyses.

In our survival models, we controlled for predetermined covariates that were potential confounders or mediators. We first adjusted for total caloric intake (continuous). Then, we controlled for known risk factors, including socioeconomic factors [years of education (continuous) (41)], lifestyle factors [marital status (never, not married currently, and married currently) and BMI (continuous)], modified Framingham Risk Score (continuous), and use of antihypertensives (yes/no).

We performed several sensitivity analyses. These included replacing baseline alcohol consumption (1) with cumulative average consumption (2, 42) from alcohol consumption at examinations 1, 2, and 3 (1969–1987); eliminating nondrinkers to reduce bias due to potential previous alcohol use; excluding heavy drinkers (≥5 servings/d) due to their greater likelihood of reduced alcohol intake during the follow-up (43); testing the association between alcohol and noncardiovascular death to address potential competing risks of noncardiovascular death; and using a truncated 30-y follow-up to reduce misclassification of alcohol exposure over the longer duration of follow-up. As previously described (42), cumulative average consumption from examinations 1–3 was calculated by using an arithmetic mean of daily alcohol intake at examinations 1, 2, and 3. If a twin was missing alcohol consumption data for a certain examination, the measurements from the available examinations were averaged.

We also conducted secondary analyses, including testing the interaction between smoking and alcohol consumption, given the observed findings from a previous twin analysis (16), and evaluating the associations between the 3 types of alcoholic beverages and outcomes. In the latter analyses, we performed within-pair analyses controlling for caloric intake, known risk factors, and average consumption of the other 2 alcoholic beverages. Given the debates around the issue of whether HDL cholesterol explains the association between alcohol intake and CAD (44), we also compared within-pair associations of overall alcohol intake with CAD mortality with and without adjustment for HDL cholesterol in models controlling for all individual risk factors. All analyses were conducted by using SAS software version 9.2 (SAS Institute). Significance levels were set at $P = 0.05$ (2-sided).

RESULTS

Characteristics of the study participants

A total of 843 twins (205 monozygotic and 191 dizygotic twin pairs, 25 monozygotic and 26 dizygotic unpaired twins) were

followed up for 41 y with a median (IQR) of 32.4 (24.1–37.4) y. There were 129 CAD deaths and 219 cardiovascular deaths during follow-up. Men with higher alcohol consumption were more likely to be current smokers and to have lower total caloric intake, higher systolic blood pressure, higher HDL cholesterol, and higher modified Framingham Risk Score ($P < 0.05$ for all trend tests) (**Table 1**). The number of deaths by cause and the number of deaths by zygosity are shown in **Table 2** and **Table 3**, respectively.

Overall associations (general population association)

After adjusting for caloric intake, per 10-g increment in either baseline or cumulative average, alcohol consumption was similarly significantly associated with lower 41-y mortality risk from CAD but not cardiovascular disease or all causes in the whole cohort (model 1 in Table 2). Further adjustment for known risk factors did not materially change the associations for cause-specific or total mortality risk (model 2 in Table 2).

Within-pair associations (control for genetic and early life environmental factors shared between co-twins)

The age-adjusted ICC for alcohol consumption in gram weight was 0.39 (95% CI: 0.27, 0.49) in monozygotic twins and 0.27 (95% CI: 0.14, 0.38) in dizygotic twins. The heritability of usual alcohol consumption was 24%, whereas the common environmental determinant accounted for 14%, suggesting potential confounding from factors shared between co-twins, including germline and early life environment and adulthood experience.

TABLE 1
Baseline characteristics of twins

		Quintile of alcohol consumption, g/d					
Characteristic	Entire cohort (N = 843)	[0, 0] (n = 218)	[1.42, 4.86] (n = 131)	[5.14, 14.15] (n = 156)	[14.2, 23.9] (n = 162)	[24.2, 106.5] (n = 176)	P-trend[1]
Alcohol consumption, g/d	8.6 (0, 21.4)[2]	0 (0, 0)	3.71 (2.43, 4.29)	8.57 (6.71, 11.4)	18.6 (18.6, 21.3)	30.9 (27.1, 44.6)	<0.0001
Total caloric intake, kcal/d	2030 ± 622[3]	2136 ± 639	1999 ± 551	2029 ± 575	2007 ± 698	1943 ± 604	0.01
Age, y	48.0 ± 3.1	48.1 ± 2.9	48.2 ± 3.1	47.6 ± 3.4	47.9 ± 3.1	47.8 ± 3.2	0.99
Smoking, n (%)							<0.0001
Never smokers	355 (42)	127 (58)	52 (40)	71 (46)	55 (34)	50 (28)	
Former smokers	82 (10)	16 (7)	11 (8)	20 (13)	13 (8)	22 (13)	
Current smokers	406 (48)	75 (35)	68 (52)	65 (41)	94 (58)	104 (59)	
Marital status, n (%)							0.27
Never married	47 (6)	8 (4)	10 (8)	14 (30)	4 (2)	11 (6)	
Married currently	51 (6)	16 (7)	3 (2)	6 (12)	8 (5)	18 (10)	
Not married currently	737 (88)	191 (89)	117 (90)	140 (19)	149 (93)	147 (84)	
Education, y	13.0 ± 2.9	12.8 ± 2.9	12.9 ± 3.0	13.4 ± 2.9	13.1 ± 2.8	13.1 ± 3.0	0.76
BMI, kg/m^2	25.8 ± 3.2	25.8 ± 3.4	26.4 ± 3.6	26.0 ± 3.3	25.2 ± 2.8	25.5 ± 2.9	0.61
Systolic blood pressure, mm Hg	128 ± 17	126 ± 17	127 ± 17	128 ± 17	129 ± 18	131 ± 15	0.002
Diastolic blood pressure, mm Hg	82 ± 11	81 ± 11	81 ± 10	81 ± 11	81 ± 11	83 ± 11	0.09
LDL cholesterol, mmol/L	3.70 ± 0.93	3.68 ± 0.83	3.76 ± 1.06	3.73 ± 0.93	3.70 ± 0.96	3.68 ± 1.01	0.39
HDL cholesterol, mmol/L	1.14 ± 0.34	1.09 ± 0.31	1.06 ± 0.31	1.11 ± 0.28	1.22 ± 0.34	1.27 ± 0.36	<0.0001
Diabetes, n (%)	42 (5)	13 (6.0)	6 (4.6)	5 (3.2)	6 (3.7)	12 (6.8)	0.23
Postload plasma glucose, mmol/L	8.67 ± 2.89	8.95 ± 2.72	8.06 ± 2.89	8.12 ± 2.84	8.84 ± 2.78	9.17 ± 3.17	0.06
Modified Framingham Risk Score,[4] unit	5.3 ± 2.2	5.0 ± 2.3	5.4 ± 2.2	5.1 ± 2.3	5.5 ± 1.9	5.6 ± 2.4	0.02
Use of antihypertensives, n (%)	33 (3.9)	5 (2.3)	8 (6.1)	9 (5.8)	2 (1.2)	9 (5.1)	0.72
Person-years	25,492	6591	3986	4918	4679	5320	
Deaths at the 41st follow-up year, n (%)							
Coronary artery disease	129 (15.3)	42 (5.0)	23 (2.7)	26 (3.1)	19 (2.3)	19 (2.3)	0.008
Cardiovascular diseases	219 (26.0)	65 (7.7)	37 (4.4)	39 (4.6)	40 (4.7)	38 (4.5)	0.06
All causes	614 (72.8)	157 (18.6)	94 (11.2)	108 (12.8)	127 (15.1)	128 (15.2)	0.49

[1]Trend test. All P values are corrected for clustering within a twin pair by using linear mixed models for continuous variables, generalized estimating equation logistic models for dichotomous variables, and repeated proportional odds model with generalized estimating equation for the 3-level smoking and marital status variables. Raw values for continuous variables are presented.

[2]Median; IQR in parentheses (all such values).

[3]Mean ± SD (all such values).

[4]The Modified Framingham Risk Score was a composite score of 7 known cardiovascular disease risk factors, including age, smoking, systolic and diastolic blood pressure, HDL and LDL cholesterol, and diabetic status. Diabetes was defined by current use of insulin or oral hypoglycemic agents or postload glucose concentration >250 mg/dL (15).

TABLE 2
Overall associations between usual daily alcohol consumption and 41-y mortality risk from specific and all causes in the entire twin cohort pooled by zygosity ($N = 843$)[1]

	Coronary artery disease		Cardiovascular disease		All causes	
	Overall association	*P* value	Overall association	*P* value	Overall association	*P* value
Deaths, *n*	129		219		614	
Model 1: Baseline alcohol, calorie adjusted	0.94 (0.89, 0.99)	0.02	0.98 (0.94, 1.01)	0.17	1.01 (0.99, 1.03)	0.45
Model 2: Baseline alcohol, multivariable adjusted[2]	0.94 (0.89, 0.98)	0.009	0.97 (0.93, 1.00)	0.06	1.00 (0.98, 1.02)	0.99
Model 3: Average alcohol,[3] multivariable adjusted[2]	0.93 (0.89, 0.98)	0.007	0.97 (0.94, 1.01)	0.11	1.00 (0.98, 1.01)	0.53

[1]Values are HRs (95% CIs) per 10-g/d increment. HRs and 95% CIs were estimated through frailty survival models to account for clustering within a twin pair. The frailty was a random effect to account for the clustering.
[2]Adjusted variables included caloric intake (continuous), BMI (continuous), marital status (yes/no), years of education (continuous), modified Framingham Risk Score (continuous), and use of antihypertensives (yes/no).
[3]Cumulative average consumption from alcohol consumption at examinations 1, 2, and 3 (1969–1987).

Such potential confounding was controlled for by conducting within-pair analyses.

The within-pair association between alcohol consumption and CAD mortality was significant before and after multivariable adjustment (models 1 and 2 in Table 3) in the whole cohort and in monozygotic twins. The multivariable-adjusted within-pair associations were significant for cardiovascular mortality for baseline alcohol consumption (model 2 in Table 3). No significant within-pair associations were observed for all-cause mortality. The within-pair association was not significantly different between monozygotic and dizygotic twins for specific and all-cause mortality (all *P*-interaction > 0.05) (Table 3).

Sensitivity analyses

We performed several sensitivity analyses to examine the robustness of the association of alcohol consumption with lower CAD mortality risk. The replacement of baseline alcohol consumption with average consumption generally yielded similar overall and within-pair associations (model 3 in Tables 2 and 3). On the basis of either baseline or average alcohol consumption,

TABLE 3
Within-pair association between usual daily alcohol consumption and 41-y mortality risk from specific and all causes in the entire twin cohort pooled and stratified by zygosity[1]

	Whole cohort ($N = 843$)		Monozygotic twins ($n = 435$; 205 pairs, 25 unpaired twins)		Dizygotic twins ($n = 408$; 191 pairs, 26 unpaired twins)	
	Within-pair association	*P* value	Within-pair association	*P* value	Within-pair association	*P* value
Coronary artery disease						
Deaths, *n*	129		68		61	
Model 1: Baseline alcohol, calorie adjusted	0.90 (0.84, 0.97)	0.008	0.88 (0.79, 0.98)	0.02	0.92 (0.82, 1.03)	0.16
Model 2: Baseline alcohol, multivariable adjusted[2]	0.90 (0.84, 0.97)	0.004	0.88 (0.80, 0.97)	0.011	0.92 (0.83, 1.02)	0.13
Model 3: Average alcohol,[3] multivariable adjusted[2]	0.92 (0.87, 0.98)	0.01	0.90 (0.82, 0.98)	0.02	0.93 (0.85, 1.02)	0.12
Test for interaction with zygosity		0.52				
Cardiovascular disease						
Deaths, *n*	219		114		105	
Model 1: Baseline alcohol, calorie adjusted	0.95 (0.90, 1.004)	0.07	0.93 (0.86, 1.002)	0.06	0.98 (0.90, 1.05)	0.52
Model 2: Baseline alcohol, multivariable adjusted[2]	0.95 (0.90, 0.997)	0.04	0.93 (0.86, 1.000)	0.049	0.97 (0.90, 1.04)	0.37
Model 3: Average alcohol,[3] multivariable adjusted[2]	0.96 (0.92, 1.00)	0.07	0.94 (0.88, 1.01)	0.07	0.97 (0.92, 1.03)	0.30
Test for interaction with zygosity		0.40				
All causes						
Deaths, *n*	614		309		305	
Model 1: Baseline alcohol, calorie adjusted	0.99 (0.96, 1.02)	0.40	0.98 (0.95, 1.02)	0.32	0.99 (0.95, 1.04)	0.79
Model 2: Baseline alcohol, multivariable adjusted[2]	0.98 (0.96, 1.01)	0.27	0.98 (0.95, 1.04)	0.29	0.99 (0.95, 1.03)	0.56
Model 3: Average alcohol,[3] multivariable adjusted[2]	1.00 (0.98, 1.02)	0.85	0.98 (0.95, 1.01)	0.20	1.01 (0.98, 1.04)	0.62
Test for interaction with zygosity		0.69				

[1]Values are HRs (95% CIs) per 10-g/d within-pair difference. HR was estimated for per within-pair 10-g/d difference in total alcohol consumption through frailty survival models to account for clustering within a twin pair. The frailty was a random effect to account for the clustering. Within-pair associations were the association between alcohol and outcomes independent of genetic and common environmental factors (i.e., early life and adult environment common to co-twins of a pair).
[2]Adjusted variables included caloric intake (continuous), BMI (continuous), marital status (yes/no), years of education (continuous), modified Framingham Risk Score (continuous), and the use of antihypertensives (yes/no).
[3]Cumulative average consumption from alcohol consumption at examinations 1, 2, and 3 (1969–1987).

we replaced the modified score with its individual components and obtained similar results (models 1 and 2 in **Supplemental Table 1**). Similarly, we repeated analyses after eliminating all nondrinkers based on either baseline or average alcohol consumption (models 3 and 4 in Supplemental Table 1) or eliminating baseline heavy drinkers (models 5 and 6 in Supplemental Table 1) with generally similar results. The multivariable-adjusted association between alcohol consumption and noncardiovascular death was not statistically significant either overall [HR (95% CI) per 10-g increment: 1.01 (1.00, 1.03), $P = 0.12$] or within-pair [HR (95% CI) per 10-g within-pair difference: 1.00 (0.97, 1.03), $P = 0.97$]. Our truncated 30-y follow-up associations tended to have greater magnitude than the 41-y follow-up results (**Supplemental Tables 2** and **3**). Finally, baseline alcohol consumption was categorized in quintiles (**Supplemental Table 4**), with further evidence of a dose-dependent inverse association of alcohol intake with CAD mortality risk.

Secondary analyses

There was no significant interaction between smoking and alcohol consumption overall or within-pair for CAD and all-cause death (all P-interaction > 0.05). The within-pair risk of cardiovascular mortality differed by smoking (P-interaction < 0.01), with an inverse association among nonsmokers (HR: 0.85; 95% CI: 0.77, 0.93, per 10-g within-pair difference) but no association among smokers (HR: 1.00; 95% CI: 0.94, 1.07).

We performed secondary within-pair analyses by beverage type (**Table 4**). After multivariable adjustment, baseline wine and beer consumption was significantly inversely associated with mortality from CAD but not cardiovascular disease or all causes. For CAD mortality risk, the partial regression coefficients for wine, beer, and spirit consumption at baseline ($P > 0.2$) or on average ($P > 0.8$) did not differ significantly, implying that ethanol itself may be most important in CAD mortality risk. The consumed amount of beverage-specific alcohol is shown in **Supplemental Table 5**.

Last, to address a possible mechanism, we repeated our within-pair analyses with and without adjustment for HDL cholesterol, controlling for all other individual risk factors. Alcohol consumption was inversely associated with risk in both cases, with only a 1% change in the partial regression coefficient, suggesting that HDL cholesterol did not mediate the association among these twins.

DISCUSSION

In this long-term follow-up of a cohort of white male twins, we found an inverse overall and within-pair association between usual alcohol consumption and mortality risk from CAD.

Our study was similar to previous general population studies in demonstrating an inverse association of alcohol consumption with the risk of death (1, 3). However, our within-pair findings provided new evidence on the association, accounting for numerous unknown and unmeasured factors shared between co-twins (15) in addition to known risk factors. These may include familial factors such as behavior, parental demographic factors and socioeconomic status shared among family members (45), and common environmental factors from sources outside the family such as cultural norms toward alcohol drinking (15). As a result of this benefit of the co-twin study design, our findings provide new evidence to support the hypothesized causal relation of alcohol consumption and CAD mortality risk, although they cannot directly prove this hypothesis (46).

Consistent with prior studies of men in the United States (2), we found that alcohol consumed from beer at baseline and cumulatively tended to be inversely associated with CAD risk, whereas a similar association was found for either baseline wine or average spirit consumption. Therefore, our results generally support the hypothesis that total ethanol per se plays the dominant role in CAD

TABLE 4
Multivariable-adjusted within-pair association between usual daily alcohol consumption by the type of alcoholic beverage and the 41-y mortality risk from specific and all causes in the entire twin cohort pooled by zygosity ($N = 843$)[1]

	Coronary artery disease		Cardiovascular disease		All causes	
	Within-pair association	P value	Within-pair association	P value	Within-pair association	P value
Deaths, *n*	129 (68 MZ, 61 DZ)		219 (114 MZ, 105 DZ)		614 (309 MZ, 305 DZ)	
Wine						
Baseline	0.73 (0.54, 0.98)	0.04	0.84 (0.67, 1.05)	0.12	0.97 (0.87, 1.09)	0.65
Average[2]	0.85 (0.68, 1.08)	0.18	0.90 (0.74, 1.09)	0.26	0.97 (0.88, 1.08)	0.58
Beer						
Baseline	0.90 (0.81, 0.99)	0.04	0.96 (0.89, 1.03)	0.22	0.97 (0.93, 1.01)	0.11
Average[2]	0.91 (0.83, 1.00)	0.06	0.96 (0.90, 1.03)	0.24	1.00 (0.96, 1.04)	0.90
Spirits						
Baseline	0.93 (0.84, 1.03)	0.18	0.95 (0.88, 1.03)	0.21	1.01 (0.96, 1.05)	0.78
Average[2]	0.90 (0.81, 1.00)	0.06	0.94 (0.88, 1.01)	0.11	1.01 (0.97, 1.04)	0.74

[1]Values are HRs (95% CIs) per 10-g/d within-pair difference. HR was estimated for per within-pair 10-g/d difference in alcohol consumption from specific alcoholic beverage through frailty survival models to account for clustering within a twin pair. The frailty was a random effect to account for the clustering. Within-pair associations were the association between alcohol and outcomes independent of genetic and common environmental factors (i.e., early life and adult environment common to co-twins of a pair). Adjusted variables included caloric intake (continuous), BMI (continuous), marital status (yes/no), years of education (continuous), modified Framingham Risk Score (continuous), the use of antihypertensives (yes/no), and alcohol consumption from the other 2 specific alcoholic beverages (continuous). DZ, dizygotic; MZ, monozygotic.

[2]Cumulative average consumption from alcohol consumption at examinations 1, 2, and 3 (1969–1987).

mortality risk, although we cannot exclude a role for the type of alcoholic beverage consumed given the observed 95% CIs.

Potential mechanisms underlying the association

The mechanisms through which alcohol consumption might reduce CAD risk have been reviewed extensively (47). Those supported by relatively strong evidence were increased HDL cholesterol and lowered fibrinogen (47). Our results and those of some cohort studies (48, 49) suggest that the role of HDL cholesterol alone might be less than previously hypothesized, although HDL cholesterol is strongly heritable, and hence the co-twin control design may not be optimal for testing this hypothesis. Other potential mechanisms in which more evidence is still needed include the improvement of insulin sensitivity through raising concentrations of adiponectin, the antiplatelet effects of alcohol consumption, and the inhibition of inflammation through the influence of factors other than fibrinogen (47).

Limitations and strengths

There are important limitations in this study. The extent of shared environment between co-twins might change over the life span. It is likely that co-twins shared environmental factors to a larger extent in early life and young adulthood as they accumulated different experiences and behaviors with aging. Co-twins could conceptually still share some adulthood environmental factors through contact, conversation, gathering together, and similar jobs; hence, our twin design might still provide some control even for unknown and unmeasured environmental factors that were shared between co-twins in adulthood.

We could not completely eliminate potential misclassification for alcohol intake; however, the resultant potential "misclassification" would generally be nondifferential with respect to the outcome and thus attenuate the association toward the null (50). Potential residual confounding could not be exclusively excluded (51). Because the well-established Framingham study protocol was used and physical examinations and in-person interviews were performed in the NHLBI Twin Study, we believe residual confounding was minimized. In addition to known risk factors unique to individuals as in other general population studies, within-pair association analyses also minimized latent residual confounding such as parental socioeconomic status, maternal factors, and genetic and shared environmental confounding. No data on physical activity were collected at baseline in the NHLBI Twin Study, but we controlled for caloric intake, baseline BMI, and lipids, all of which were known to be associated with physical activity, with a robust result.

Our overall associations, equivalent to those in a general population, were tested in a cohort born between 1917 and 1927. Cohort effects are generally conceptualized as variation in the risk of a health outcome according to year of birth (52). Similar to prospective studies in general populations, we cannot exclude the possibility of the birth cohort bias for the overall associations (i.e., that our overall association results are not necessarily generalizable to cohorts born outside of the 1917–1927 period). By contrast, by comparing co-twins with each other, our within-pair association analysis naturally controlled for internal cohort effects due to identical birth dates for co-twins.

The NHLBI Twin Study did not collect data on former drinking history. However, when we excluded all abstainers, our results remained robust. Our results also remained robust with exclusion of heavy drinkers and with use of either baseline or repeatedly updated alcohol intake.

Changes in other risk factors and treatment might have occurred during the follow-up period that might attenuate the observed associations (53); if so, our results might underestimate the true association of alcohol with lower CAD mortality. Similarly, alcohol use may have changed over time. The correlation for total ethanol intake between 2 repeated measures was 0.71 over 10 y and 0.67 over 18 y in our study, comparable to or higher than commonly used clinical biomarkers related to long-term CAD and cardiovascular disease (54). Given the limited variability in alcohol intake over time, which is expected in a cohort enrolled during middle adulthood (55), and limited sample size in our study, we did not study within-person change in intake over time. Our subjects were white male twins only. Although the within-pair associations among monozygotic twins fully controlled for shared genes and inherited epigenetic modifications, caution should nevertheless be taken in generalizing our findings to females and other racial groups. Equally, this cohort did not contain large numbers of very heavy drinkers, and the linear association that we observed is unlikely to generalize to heavy alcohol consumers.

Our study had several advantages. Compared with traditional observational epidemiologic studies, the study design and our modeling strategy enabled the evaluation of general population-alike associations (overall association) and the within-pair associations. The within-pair associations were free of shared genetic and common environmental influences. Because each pair member shares the same birth date and co-twins are subject to the same age and period changes during follow-up, within-pair associations are controlled for age, cohort, period effects, and secular trend as unmeasured (latent) environmental factors. By considering alcohol consumption as a continuous instead of a categorical variable, we increased the number of discordant twin pairs for the exposure to alcohol and, consequently, greater statistical power. The well-established NHLBI Twin Study is the longest prospective twin study that was originally designed for the elucidation of genetic and environmental factors in cardiovascular diseases in the United States.

In conclusion, we observed an inverse association between usual alcohol consumption, measured as midlife intake or cumulative average intake over 18 y, and long-term CAD mortality risk that was independent of traditional risk factors, germline, and common environmental factors shared by twins.

The authors' responsibilities were as follows—JD and TR: had full access to all the data in the study and took responsibility for the integrity of the data; JD and KJM: designed the research; and JD, KJM, REK, GES, and TR: provided critical revisions to the manuscript for important intellectual content, satisfied the authorship criteria of the International Committee of Medical Journal Editors, conducted research and provided essential materials, and read and approved the final manuscript. The major part of this work was performed when JD worked at Indiana University School of Public Health–Bloomington. None of the authors reported any conflicts of interest.

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