Drinking Frequency, Mediating Biomarkers, and Risk of Myocardial Infarction in Women and Men

Kenneth J. Mukamal, MD, MPH, MA; Majken K. Jensen, MSc; Morten Grønbæk, MD, PhD, DMSc; Meir J. Stampfer, MD, DrPH; JoAnn E. Manson, MD, DrPH; Tobias Pischon, MD, MPH; Eric B. Rimm, ScD

Background—The associations of drinking frequency and quantity with risk of myocardial infarction have not been studied among women, and the degree to which specific risk factors mediate the inverse association of drinking frequency with risk of myocardial infarction is uncertain.

Methods and Results—We conducted nested case-control studies of 32 826 women enrolled in the Nurses Health Study followed up from 1990 to 1998 and 18 225 men enrolled in the Health Professionals Follow-Up Study followed up from 1994 to 2000. A total of 249 women and 266 men with incident myocardial infarction were matched on age, smoking, and date of entry to 498 female and 532 male control participants. We determined the risk of myocardial infarction related to frequency and quantity of alcohol intake and the change in risk before and after adjustment for putative cardiovascular risk factors. Among both women and men, drinking frequency tended to be associated with lower risk of myocardial infarction, with the lowest risks among those who drank 3 to 7 days per week. Further adjustment for levels of high-density lipoprotein cholesterol, hemoglobin A1c, and fibrinogen attenuated 75% of the association of frequent drinking with risk among women and fully attenuated the association among men.

Conclusions—Alcohol intake at least 3 to 4 days per week is associated with a lower risk of myocardial infarction among women and men, an association apparently attributable to the relationship of alcohol with HDL cholesterol, fibrinogen, and hemoglobin A1c. Because the effects of alcohol on HDL cholesterol, fibrinogen, and insulin sensitivity have been confirmed in randomized trials, our findings support the hypothesis that the inverse relation of alcohol use and myocardial infarction is causal. (Circulation. 2005;112:1406-1413.)

Key Words: alcohol ■ women ■ men ■ epidemiology ■ myocardial infarction

Substantial epidemiological evidence links moderate alcohol consumption with a lower risk of myocardial infarction (MI). This evidence includes results from geographic comparisons,1 large cohort studies2–5 and several meta-analyses.6–9 However, the role of pattern of alcohol consumption has not been well studied. We recently found that frequency of alcohol intake was the primary determinant of lower risk of MI among men,10 but we know of no comparable studies in women, who differ from men both in their risk of MI and their metabolism of ethanol.11

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The lower risk of MI among moderate drinkers has been attributed in great part to their higher levels of high-density lipoprotein (HDL-C).12 HDL-C is strongly associated with lower risk of MI in epidemiological studies13 and appears to have beneficial vascular effects when augmented experimentally.14,15 In both the Honolulu Heart Program and Multiple Risk Factors Intervention Trial, adjustment for HDL-C levels attenuated the estimated effect of alcohol by approximately 50%, which suggests that approximately half of the benefit attributable to moderate drinking was mediated by HDL-C.16,17 We know of no study that has formally evaluated the role of other factors that could mediate the lower risk of MI among moderate drinkers.

The role of mediating factors in the inverse association of moderate drinking and risk of MI is particularly important because no long-term randomized trial has been performed to...
confirm this association, nor is one likely in the near future. However, multiple short-term randomized trials of alcohol administration in humans demonstrate effects of moderate alcohol consumption on several potential mediators. For example, a meta-analysis of more than 40 such trials confirmed that consumption of 30 g of alcohol daily (about 2 standard drinks) raises levels of HDL-C by 0.10 mmol/L and lowers levels of fibrinogen by 0.22 μmol/L.\(^{18}\) Moderate alcohol use also improves insulin sensitivity\(^{19,20}\) and possibly markers of inflammation\(^{21}\) in experimental trials. Evidence that biomarkers directly affected by alcohol mediate the relation of alcohol and risk of MI would support the hypothesis that this relation is causal.

To address these issues, we studied the relation of alcohol consumption to risk of MI among participants in 2 large, well-characterized cohort studies, women enrolled in the Nurses Health Study (NHS) and men enrolled in the Health Professionals Follow-Up Study (HPFS).\(^{22}\) These studies include prospectively collected information on drinking patterns, dietary and lifestyle factors, and serum levels of biomarkers. In these analyses, we assessed the associations of quantity and frequency of alcohol consumption with risk of MI among both women and men and the role of a wide array of potentially mediating biomarkers in these associations.

**Methods**

**Study Population**

The NHS cohort was established in 1976. The study population consists of 121,700 married female registered nurses aged 30 to 55 years residing in 1 of 11 larger US states. Women have received follow-up questionnaires biennially to update information on exposures and newly diagnosed illnesses. Since 1980, participants have updated information on diet, alcohol, and vitamin supplements through a food frequency questionnaire (FFQ) every 4 years.

The HPFS began in 1986, when 51,529 male health professionals 40 to 75 years of age completed the initial 6-page HPFS questionnaire. The population includes 29,683 dentists, 3745 optometrists, 2218 osteopathic physicians, 4185 pharmacists, 1600 podiatrists, and 10,098 veterinarians. Biennial follow-up has mirrored the NHS.

**Assessment of Biomarkers**

Blood samples were requested from all active participants and collected from 32,826 NHS members in 1989 to 1990 and 18,225 HPFS members in 1993 to 1994. With the exception of a modestly lower prevalence of smoking, those who returned blood samples did not differ substantially from those who did not in both cohorts, including average alcohol intake of 6.5 versus 6.3 g/d among women and 12.8 versus 12.5 g/d among men. Participants underwent local phlebotomy and returned samples to our laboratory via overnight courier. On arrival, whole blood samples were centrifuged and stored in cryotubes as plasma, buffy coat, and red blood cells in the vapor phase of liquid nitrogen freezers.

The measurement of biomarkers in this sample has been described previously. The markers generally showed excellent stability and reproducibility during simulated transport and storage.\(^{23}\) Homocysteine levels were only assayed among women, and adiponectin (a plasma protein associated with insulin sensitivity\(^{24,25}\)) levels were only assayed among men. Candidate biomarkers assessed in both women and men included LDL cholesterol, triglycerides, total cholesterol, apolipoprotein B, soluble tumor necrosis factor receptors 1 and 2 (sTNFR1 and sTNFR2), interleukin-6, high-mobility group A1c (HbA1c), soluble intercellular adhesion molecule-1, soluble vascular cell adhesion molecule-1, high-sensitivity C-reactive protein,\(^{26}\) lipoprotein(a), and fibrinogen.

**Assessment of Alcohol Consumption**

We assessed diet with a 131-item semiquantitative FFQ administered every 4 years that included separate items for beer, white wine, red wine, and liquor, as described previously.\(^{10}\) We previously validated estimated alcohol consumption against two 1-week dietary records collected approximately 6 months apart from 173 NHS participants and 136 HPFS participants residing in eastern Massachusetts.\(^{27}\) With Spearman correlation coefficients between these 2 measures of 0.90 in women and 0.86 in men. Estimated mean alcohol intake among men was 12.5 g/d with dietary records and 12.5 g/d with the FFQ; estimated mean intake among women was 9.0 g/d with both methods. In 1986 and 1988, NHS and HPFS participants reported the number of days per week that they typically drank any form of alcohol. The correlation coefficient between this measure and diet records among men was 0.79.\(^{28}\)

In these analyses, we used average alcohol intake from the 1990 FFQ among women and the 1994 FFQ among men (ie, at the time of the blood draw) and frequency of alcohol intake assessed in 1988, using previous assessments when missing data occurred.

**Assessment of MI During Follow-Up**

We used World Health Organization criteria for MI, which require typical symptoms and either diagnostic ECG changes or elevated cardiac enzymes (including cardiac-specific troponin).\(^{29}\) Fatal MI included fatal cases defined by World Health Organization criteria with hospital records, or if coronary heart disease was listed as the cause of death on the death certificate, was the most plausible cause, and evidence of previous coronary heart disease was available. We included sudden cardiac deaths, defined as death within 1 hour of symptom onset with no previous serious illness and no other plausible cause. Physicians reviewing medical records were unaware of participants’ reported alcohol intake.

**Case-Control Sampling**

We performed parallel nested case-control studies within the samples of women and men who provided blood samples. In the NHS, we identified 249 women free of cardiovascular disease or cancer in 1990 who sustained an incident MI before June 30, 1998. In the HPFS, we identified 266 men free of cardiovascular disease in 1994 who sustained an incident MI before January 31, 2000. Of the 266 cases in HPFS, 196 had nonfatal MI and 70 had fatal CHD; the corresponding numbers in the NHS were 212 nonfatal cases and 37 fatal events.

For each incident case of MI, we randomly selected 2 women or men free of cardiovascular disease matched on age (in 5-year increments), smoking (in 5 categories), and month of blood return using risk-set sampling.\(^{10}\) In the NHS, matching criteria also included fasting status and reported problems with blood drawing. In analyses of drinking frequency, we deleted 4 women (1 case and 3 controls) but no men who were missing this information.

**Statistical Analysis**

We calculated ORs from conditional logistic regression models as measures of relative risk, stratifying on the matching variables. In multivariable analyses, we additionally adjusted for body mass index (in quintiles), parental history of MI at or before age 60 years, diabetes, hypertension, hypercholesterolemia, physical activity (in 5 categories), aspirin use, supplemental use of vitamin E, energy intake (in quintiles), and energy-adjusted intake of saturated fat, trans fatty acids, omega-3 fatty acids, folate, and dietary fiber (each in quintiles). Nutritional variables, anthropomorphic measures, aspirin use, and self-reported cardiovascular risk factors have been validated.\(^{31}\) We also adjusted for current hormone replacement therapy use among women. We used covariate information from the time of the blood draw, defined as 1994 in the HPFS and 1990 in the NHS. We used information from previous questionnaires when covariate data from the time of blood draw were missing. We conducted tests of linear trend across increasing categories of alcohol consumption by treating the midpoints of consumption in categories as a continuous
variable; we squared this variable after centering to test quadratic trend.

We tested potential mediators of the association of alcohol use and risk of MI by comparing the regression coefficients associated with drinking frequency from multivariable models with coefficients from models additionally adjusted for each mediator in quintiles, consistent with previous work.16 The regression coefficients are the natural log of the ORs for each level of intake and are scaled linearly. Because these analyses require a single coefficient to describe the primary exposure, we incorporated drinking frequency as a continuous variable to reflect our findings in men and to include the full range of data; sensitivity analyses that incorporated drinking frequency in categories (that better specify the model form for women) are shown in the online-only Data Supplement Table. We also assessed the effects of adjustment for groups of mediators, including lipids (HDL-C, LDL cholesterol, total cholesterol, triglycerides, lipoprotein(a), and apolipoprotein B), inflammatory markers (interleukin-6, soluble intercellular adhesion molecule-1, soluble vascular cell adhesion molecule-1, sTNFR1, sTNFR2, and C-reactive protein), and markers implicated in randomized trials (HDL-C, fibrinogen, and HbA1c, or adiponectin).

To maintain consistent sample sizes in the NHS, we assigned indicator variables when levels of a given mediator were missing (n=7 to 49). Analyses that instead deleted women with missing mediators or that assigned such women median population values yielded substantially similar results that are not shown here. We also assigned indicator variables for 90 women with missing triglyceride levels; analyses that used the population median gave similar results, and both approaches yielded results consistent with our previous findings on triglycerides and risk of MI.32 Only 4 men in the HPFS were missing values for any mediator, and hence these men were deleted only from analyses that included the missing mediator.

To determine whether the magnitude with which biomarkers appeared to mediate the relationship of alcohol intake with risk of MI was consistent with known effects of alcohol from randomized trials,14 we determined the relation of alcohol intake with candidate biomarkers across the range of drinking frequency in linear regression models, adjusting for the same covariates as in logistic regression models. We used robust variance methods to accommodate deviations from normality of the conditional outcome variable.33

Results

Characteristics of Cases and Controls

Table 1 shows the characteristics of case and control participants in the HPFS and NHS; the characteristics of these participants according to alcohol use have been reported previously.5,10 As expected, traditional cardiac risk factors were more common, HDL-C levels were lower, and LDL cholesterol levels were higher among cases than among controls. The age-adjusted correlations between drinking frequency and average quantity of use were 0.75 among women and 0.64 among men, with little difference between cases and controls. There was little variability in the amount of alcohol consumed per drinking day across the range of frequency of use, with median intakes of 18.2 to 20.1 g (~1.5 drinks) per drinking day among control women and 22.4 to 26.2 g (~2 drinks) per drinking day among control men.

Drinking Pattern and Risk of MI

Figure 1 shows the relative associations of average alcohol consumption and drinking frequency with risk of incident MI in the NHS. Figure 2 shows similar results in the HPFS. For both men and women, drinking frequency was associated with risk of MI.

Among men, there was a significant graded inverse relation of MI risk with drinking frequency (P trend 0.04), with the lowest risk among men who drank 5 to 7 days per week. In previous analyses of the entire HPFS cohort,10 the risks were similar among men who drank 3 to 4 and 5 to 7 days per week.

Among women, the risk was lowest among those who drank 3 to 4 days per week, with a slightly higher risk among those who drank more frequently. We did not find significant results in a test for quadratic trend in drinking frequency (P=0.13) or in a comparison of intake 3 to 4 versus 5 to 7 days per week (P=0.38) among women; the corresponding probability value from a test for linear trend was 0.02. The relationship between drinking frequency and MI risk was generally similar among participants who did and did not take aspirin (data not shown).

Mediators of the Relation of Drinking Frequency and Risk of MI

We added potentially mediating biomarkers one at a time (and then in groups) into multivariable regression models to assess the magnitude with which each biomarker or group of biomarkers changed the association of frequent drinking with risk of MI (Table 2 and Data Supplement Table). Among women, multivariable analysis suggested an OR of 0.76 (95% CI 0.59 to 0.97), which corresponds to a regression coefficient of −0.28 per drinking day. HDL-C alone accounted for 36% of this association, and HbA1c alone accounted for a similar proportion. Other biomarkers only modestly attenuated the regression coefficient. When HDL-C, HbA1c, and fibrinogen were included together, these 3 biomarkers explained 75% of the inverse association of frequent alcohol intake and risk of MI.

Among men, the OR associated with frequent intake of alcohol (in drinking days per week) was 0.86 (95% CI 0.74 to 1.00), which corresponds to a regression coefficient of −0.15. Further adjustment for HDL-C alone dropped the regression coefficient to −0.07, which suggests that approximately half of the inverse association was attributable to HDL-C among men. Individual adjustment for adiponectin, fibrinogen, triglycerides, or HbA1c in the multivariable models attenuated the association by ~30%. Adjustment for the combination of HDL-C, fibrinogen, and HbA1c fully attenuated the regression coefficient in men.

Because the relationship of drinking frequency and risk was not linear among women, we performed sensitivity analyses that assessed mediators in a comparison of consumption 3 or more days per week to consumption <1 day per week, reflecting the plateau in risk at 3 to 4 days per week (Data Supplement Table). In these analyses, adjustment for the combination of HDL-C, fibrinogen, and HbA1c reduced the association of frequent drinking with risk by 61% among women and eliminated it among men (adjusted coefficient 0.01).

Because the association of frequent drinking and risk of MI differed little between models that included only matching factors and larger multivariable models (Table 2 and Data Supplement Table), we also examined potentially mediating biomarkers in models with only matching factors. These models with fewer covariates again yielded very similar results. Adjustment for HDL-C, fibrinogen, and adiponectin...
as an alternate measure of insulin sensitivity also directly affected by alcohol and yielded results similar to HDL-C, fibrinogen, and HbA1c, as did the replacement of fibrinogen with C-reactive protein (which is also lowered by alcohol use).

Because frequency and overall quantity of drinking were closely correlated among women, we examined the relationship of quantity of intake with risk of MI. Compared with abstention, intake of at least 5.0 g/d was associated with a relative risk of 0.56 (95% CI 0.34 to 0.92). Adjustment for HDL-C alone reduced the association of this quantity of drinking with risk by 24% compared with 55% for the combination of HDL-C, fibrinogen, and HbA1c.

Relation of Alcohol Use to Biomarkers Implicated in Randomized Trials

We next assessed the relation of drinking frequency with levels of the 3 biomarkers for which randomized trials provided the strongest evidence for an effect of alcohol: HDL-C, fibrinogen, and HbA1c (Table 3). Across the distribution of alcohol use, we found a range in HDL-C levels of roughly 0.2 to 0.3 mmol/L, consistent with the range expected from clinical trials. The differences in HbA1c levels were also within a credible range, with levels among frequent drinkers 0.1 to 0.2 percentage points lower among frequent drinkers than among abstainers. The distribution in fibrinogen levels (which appeared to mediate less of the association of alcohol...
with risk) was somewhat broader, with differences across drinking categories of 0.2 to 1.0 μmol/L.

Discussion

In this nested case-control study, frequency of alcohol intake was associated with lower risk of MI among both women and men. Among women, HDL-C, fibrinogen, and HbA1c (a marker of glucose tolerance) accounted for 75% of the association. Among men, this association appeared to be virtually entirely attributable to these factors.

The results of the present study provide support for a causal relation between alcohol use and risk of MI, a relation that would be difficult to test in a long-term randomized trial. We found associations between alcohol use and candidate intermediates that were of credible magnitude and that appeared to account for most or all of the observed alcohol-MI relation in statistical models.

In both this and previous analyses, we found drinking frequency to be inversely associated with risk of MI, but not necessarily in a linear fashion. Among women in the present study and men in a larger previous study, there appeared to be a clear threshold in risk at 3 to 4 drinking days per week, a level of consumption that is also consistent with a previous meta-analysis of alcohol use and nonfatal MI. Interestingly, Dorn and colleagues recently found that greater drinking frequency had a strong inverse cross-sectional association with central adiposity among both women and men, whereas a higher quantity of intake per drinking day had a positive relation. Because central adiposity is linked to low levels of HDL-C, high levels of inflammation-sensitive proteins (including fibrinogen), and glucose intolerance (because all are part of the metabolic syndrome), it is intriguing to hypothesize that alcohol use could act early in the pathway that leads to these linked abnormalities. Indeed, average alcohol intake has been inversely associated with prevalence of metabolic syndrome in some studies. However, other direct mechanisms may contribute to the effect of alcohol on HDL-C levels.

We found inverse relations of alcohol use and risk of MI among both men and women, although the association was generally stronger among women. At the same time, measured biomarkers accounted for a larger proportion of the association among men. These observations could be due to chance or to a greater degree of residual confounding among women. However, they may also suggest that alcohol has particular effects on cardiovascular risk markers that are stronger among women than men and for which we did not have data. For example, small, dense LDL particles may be particularly linked to risk of coronary heart disease among women, and limited experimental and epidemiological evidence suggests that alcohol use could increase levels of larger LDL particles via effects on lipoprotein lipase.

The present results provide indirect support for the hypothesis that ethanol itself, rather than nonalcoholic components of some alcoholic beverages, is the factor responsible for the relation of moderate drinking and risk of MI. Because this

Figure 1. Adjusted ORs for incident MI among women according to average daily alcohol intake (A) or frequency of alcohol intake (B). Probability values shown are from tests of linear trend. The ORs are conditioned on matching factors of age, smoking, and date of blood sample return and adjusted for body mass index, parental history of MI, diabetes, hypertension, hypercholesterolemia, physical exertion, aspirin use, supplemental use of vitamin E, energy intake, and energy-adjusted intake of saturated fat, trans fatty acids, omega-3 fatty acids, folate, and dietary fiber. I-bars indicate 95% CIs.

Figure 2. Adjusted ORs for incident MI among men according to average daily alcohol intake (A) or frequency of alcohol intake (B). Legend as in Figure 1.
relation appears to be mediated predominantly by biomarkers linked to ethanol intake itself, it is unlikely that other components influence the relation to an important degree. However, because wine is the most frequently consumed beverage among women but not men, it is possible that nonalcoholic constituents of wine may also contribute to the greater risk reduction found in women.

Specific limitations of the present study warrant discussion. As in any observational study, our results could be influenced by differences between participants in factors other than alcohol consumption for which we did not adjust. In this study, we adjusted for age, smoking, diet, exercise, body mass index, aspirin use, and cardiovascular risk factors, and our populations were homogeneous with respect to occupational class and sex. To have influenced our results on mediators, any remaining confounder would need to be associated with both alcohol consumption and risk of MI and generally be unrelated to the other covariates in our multivariable models, yet still associated with a measured biomarker. In such a scenario, inclusion of that measured biomarker in our models would effectively partially adjust for the uncontrolled confounder and not solely test its mediating potential. The fact that the magnitude of the relations between the 3 key biomarkers and drinking frequency was comparable to the magnitude seen in randomized trials argues against (but cannot disprove) this possibility.

We studied 2 groups of predominantly white health professionals, and hence, we cannot necessarily generalize our results to other populations with different educational levels, incomes, or distributions of race and ethnicity. However, the relationship of alcohol intake with risk of MI reported here is quite similar to that found in a wide variety of populations.7

Although we documented >500 incident cases of MI among >50 000 individuals in the present study, our power to detect differences, particularly for individual categories of consumption, was limited in some cases. Likewise, drinking
Several of the mediators that we studied correlate and interact with each other. For example, although fibrinogen is part of the thrombotic pathway, it also clusters with other inflammation-sensitive proteins. Thus, adjustment for fibrinogen may, in essence, partially adjust for more traditional inflammatory markers, and adjustment for C-reactive protein instead of fibrinogen led to similar results. This does not alter the fact that moderate drinking influences several biomarkers directly, but it suggests that the causal pathway between alcohol use and risk of MI may be more complex than it appears at first glance. We also made the conservative decision to adjust for prevalent diabetes, but this may have led us to underestimate both the association of drinking frequency with risk of MI and the mediating effect of insulin sensitivity, because moderate drinking is associated with a lower risk of incident diabetes in these cohorts.50,51

In summary, we found inverse relations of frequency of alcohol intake with risk of MI that were at least as strong as the relation of average alcohol use with MI risk. Levels of HDL-C, HbA1c, and fibrinogen, which have been causally linked to frequent alcohol use in randomized trials, appeared to mediate the bulk of the alcohol-MI relation. The present results provide support for the hypothesis that the observed relationship of alcohol intake and risk of MI is causal, although definitive confirmation of this will require long-term, randomized outcome trials. In the interim, clinical decisions about appropriate alcohol use should ultimately reflect individual susceptibility to the entire balance of risks and benefits attributable to alcohol use.

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Disclosure

Eric Rimm has received honoraria for occasional speaking engagements at research conferences from industry-related organizations (the Distilled Spirits Council and the National Beer Wholesalers Association).

References


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