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THE ROLE OF HIGH DENSITY LIPOPROTEIN FOR ALCOHOL INTAKE AND MYOCARDIAL INFARCTION: RESULTS FROM TWO PROSPECTIVE COHORTS

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ABSTRACT

Myocardial infarction (MI) is the most common form of coronary heart disease, which is by far the most common cardiovascular condition. Despite the improvement and increased use in evidence-based therapies and lifestyle interventions, it remains the number one cause of morbidity and mortality worldwide. Alcohol intake has both a beneficial and a harmful influence on atherosclerosis, which is the main pathological change in MI. Moderate alcohol intake has been found to be associated with a lower risk for MI in numerous epidemiological studies, and it is hypothesized that this protective effect is mainly mediated through high-density lipoprotein cholesterol (HDL-C) concentration. However, controversial results have been generated from epidemiological studies on this topic and the role of HDL-C concentration being a sufficient predictor for cardiovascular disease has been challenged.

The objective of the first study in this dissertation was to determine the association between total alcohol intake and type of alcohol containing beverage, and 6-year (2006-2012) longitudinal change in HDL-C concentrations in a community-based cohort. In this study, we included 71,379 Chinese adults (mean age 50±11 yr.) who were free of cardiovascular diseases and cancer, and did not use cholesterol-lowering agents during follow-up. Alcohol intake was assessed by a questionnaire in 2006 (baseline) and participants were categorized as never, past, light (women: 0-0.4 servings/d, men: 0-0.9 servings/d), moderate (women: 0.5-1.0 servings/d, men: 1-2 servings/d), and heavy drinkers (women: >1.0 servings/d, men: >2 servings/d). HDL-C concentrations were measured in 2006, 2008, 2010, and 2012. We used generalized estimating equation models to examine the associations between baseline alcohol intake and change in HDL-C concentrations, adjusting for age, sex, smoking, physical activity, obesity, hypertension, diabetes, liver function and C-reactive protein concentrations. We observed an umbrella-shaped association between total alcohol consumption and changes in HDL-C concentration. Compared

with never drinkers, past, light, moderate and heavy drinkers, respectively, experienced a 0.012 (95% CI: 0.008, 0.016), 0.013 (95% CI: 0.010, 0.016), 0.017 (95% CI: 0.009, 0.025), and 0.008 (95% CI: 0.005, 0.011) mmol/L per year slower decrease in HDL-C ($P < 0.0001$ for all) after adjusting for potential confounders. Moderate alcohol consumption was associated with the slowest increase in total cholesterol/HDL-C and triglyceride/HDL-C ratios. We observed a similar association between hard liquor consumption and HDL-C change. In contrast, greater beer consumption was associated with slower HDL-C decreases, in a dose-response manner.

The objective of the second study was to test the hypothesis that the lower risk of myocardial infarction (MI) associated with alcohol intake is mediated by raising HDL-C concentration. This study included 81,253 Chinese men and women (mean age: 51±12 yr.) from the Kailuan Study who were free of cardiovascular disease in 2006 (at baseline) and were followed up to Dec. 2016. At baseline, alcohol consumption was assessed via a questionnaire and the concentration of HDL cholesterol was measured. Incident MI at follow up was a first MI event, confirmed by medical record review. Multivariable Cox regression was used to model the association between habitual alcohol intake and risks of MI, adjusting for potential covariates including age, sex, education, monthly income, occupation, smoking status, physical activity, body mass index, waist circumferences, hypertension, diabetes and total cholesterol. Mediated effect through HDL cholesterol was assessed using a causal mediating analysis (SAS macro). During an average of 9.6 years of follow-up, we documented 1088 incident cases. The adjusted hazard ratio (HR) for MI was 0.74 (95% confidence interval (CI): 0.60, 0.91), 0.80 (95%CI: 0.56, 1.16), 0.56 (95%CI: 0.45, 0.70) for light, moderate, and heavy alcohol drinkers compared with non-drinkers. The ratio changed very slightly after further adjustment of HDL cholesterol concentration. Mediation analysis showed that HDL cholesterol concentrations mediated a small, non-significant proportion (Proportion mediated ~2%) of the association between alcohol and MI.

The objective of the third study was to investigate whether and to what extent the association between alcohol intake and incident myocardial infarction (MI) is mediated through HDL-C concentration, HDL particles (HDL-P) concentration, and apoA-I concentrations. A total of 6,683 participants from the Multi-Ethnic Study of Atherosclerosis (MESA) were included in the analysis. Alcohol consumption was assessed via a questionnaire at baseline (exam 1). HDL-C concentrations and HDL-P were both measured at exam 1. Cox regression was used to model the association of habitual alcohol intake and risk for MI before and after adjusting HDL-C and HDL-P, in addition to adjustment of all potential covariates. Mediated effects through HDL-C, and HDL-P were estimated using the causal mediation analysis. After a median of 8 years follow-up, 171 incident MI cases were documented. Higher alcohol intake was associated with a lower risk for incident MI (P for trend =0.039). The relation between alcohol intake and MI was slightly attenuated by adjustment of HDL-C and moderately by HDL-P. Mediation analysis showed no mediating effect of HDL-C (HR^{NIE}: 0.98; 95%CI: 0.94, 1.02; P-value=0.26), whereas HDL-P slightly mediated the association of habitual alcohol intake and MI (HR^{NIE}: 0.95, 95%CI: 0.90, 0.99; P-value=0.027). The proportion of the total effect of alcohol on MI mediated by HDL-P was 15.8%.

In conclusion, we found that moderate alcohol consumption was associated with slower HDL-C decreases; however, the type of alcoholic beverage was differently associated with the change in HDL-C concentrations. Alcohol consumption was associated with a lower risk for MI incidence in both Chinese and MESA cohorts. Our results suggest that the benefits on MI associated with moderate alcohol consumption are not related to the effects of alcohol on HDL cholesterol. The lower risk of MI related to alcohol intake appears to partially work through increasing HDL-P. The mechanism for much of alcohol's effect to reduce MI risk remains unexplained. This suggests that HDL-P can be a target for MI

prevention, however the mediating effect of HDL-P is very moderate. Future studies arewarranted to confirm our finding regarding HDL-P and further evaluate if HDL-P concentration may be a surrogate biomarker reflecting the anti-atherogenic HDL function to decide if HDL-P can be used as a new risk predictor and intervention target for CVD.

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Chapter 1

Introduction

Background

Myocardial infarction (MI) is the most common form of coronary heart disease. Improvement and increased use in evidence-based therapies and lifestyle interventions have promoted reductions in mortality from coronary heart disease in the past decades (1, 2). However, MI remains the number one cause of morbidity and mortality worldwide. In 2015, it is estimated that there were 7.29 million acute MIs worldwide (3). In the US, the estimated annual incidence of MI is 805,000 including 605,000 new attacks and 200,000 recurrent attacks (2) and it causes more than 111,777 deaths. The estimated cost of MI is tremendous; in 2013, MI was the most expensive condition treated in US hospitals with a hospital cost of US $12.1 billion (4). Therefore, it is important to identify and examine modifiable risk factors for disease control and prevention.

Alcohol intake is one risk factor that has both a beneficial and a harmful influence on atherosclerosis, which is the main pathological change in MI. On one hand, high-dose alcohol consumption induces arterial damage in coronary and peripheral limb arteries, and increases individual cardiovascular risk factors such as hypertension, hypertriglyceridemia, which are thought to be responsible for the increased cardiovascular risk (5, 6). On the other hand, light-to-moderate alcohol consumption is generally thought to reduce the risk of cardiovascular disease, particularly atherosclerosis-induced disease including coronary disease and ischemic stroke, through elevated lipid levels, accelerated glucose metabolism, and anti-inflammatory effects (7-9).

Because of the well-established inverse relationship between HDL-C and cardiovascular disease (CVD) from a large number of epidemiological studies and also from intervention studies alcohol has been observed to increase HDL-C (10-13), the cardioprotective effect of alcohol is thought to be primarily through its effect on HDL-C (14). However, controversial results have been generated from epidemiological studies on this topic (14, 15). Moreover, data from human genetic data and clinical trials have challenged the role of HDL-C being a predictor for cardiovascular disease (16) and thus raised doubts in the causal relationship between alcohol intake and risk of MI. Therefore, the interrelation among alcohol, HDL and MI remains unclear and further investigations are warranted.

Objectives

Three primary objectives were investigated for partial fulfillment of this dissertation research project:

Objective 1: To examine the association between habitual alcohol intake and HDL-C concentration over time in a community-based cohort

Objective 2: To examine if HDL-C concentration mediates the association between habitual alcohol intake and risk of MI in the Kailuan cohort

Objective 3: To examine the mediating role of HDL-C, HDL particle concentrations (HDL-P) and apolipoproteinA-Ⅰ for the association between habitual alcohol intake and risk of MI in the Multi-Ethnic Study of Atherosclerosis cohort

Data from two cohorts will be used for the objectives mentioned. For objective 1 and 2 the Kailuan Study was used. Kailuan Study (Chinese Clinical Trial Registry number: ChiCTR-TNRC-11001489) is an on-going prospective cohort study conducted in the Kailuan community

in Thangshan City in China. As detailed previously (17, 18) , 101,050 participants (81,110 men and 20,400 women, age range: 18-97 yr.) were recruited from the Kailuan community in 2006-2007 (i.e. baseline). At recruitment, all participants underwent a standardized questionnaire interview and physical examination and laboratory assessment. They were followed up biennially on the above measurement through 2016 December. Detailed exclusion criteria are explained in chapter 3 and chapter 4.

For objective 3, the Multi-Ethnic Study of Atherosclerosis (MESA) was used. It is a prospective cohort study designed to investigate the prevalence, risk factors, and progression of subclinical cardiovascular disease in a multi-ethnic cohort in the United States. The study design and methods have been described previously (19). Briefly, from 2000 to 2012, 6814 non-pregnant participants aged 45-84 y were recruited from 6 US communities: Forsyth County, NC; New York, NY; Baltimore, MD; St Paul, MN; Chicago, IL; and Los Angeles, CA. Each site recruited an approximately equal number of men and women, according to the pre-specified age, gender, and race/ethnicity proportions. Baseline exclusion criteria included self-reported cardiovascular diseases (heart attack, angina, stroke, transient ischemic attack, heart failure, current atrial fibrillation, history of CVD related procedures including coronary artery bypass grafting (CABG), angioplasty, valve replacement, pacemaker or defibrillator implantation and any other surgery on the heart or arteries), cancer, weight over 300 pounds, and cognitive inability.

Dissertation content and format

This dissertation begins with a review of the literature examining the associations between 1) alcohol intake and risk of MI 2) HDL and cardiovascular risk and 3) Alcohol intake, HDL and cardiovascular risk. Chapter 3 reported the results of objective 1. Chapter 4 presents the findings from objective 3, using mediation analysis to investigate the mediating role of HDL-C in

the Kailuan cohort. Chapter 5 addresses the mediating role of HDL-C, HDL-P, and apoA-Ⅰ in the Multi-Ethnic Study of Atherosclerosis. The content presented in Chapter 3 has been published in the *American Journal of Clinical Nutrition*. Tables, figures and references are located at the end of each chapter.

References

1. Reed GW, Rossi JE, Cannon CP. Acute myocardial infarction. Lancet (London, England) 2017;389(10065):197-210. doi: 10.1016/s0140-6736(16)30677-8.
2. Benjamin EJ, Muntner P, Alonso A, Bittencourt MS, Callaway CW, Carson AP, Chamberlain AM, Chang AR, Cheng S, Das SR, et al. Heart Disease and Stroke Statistics-2019 Update: A Report From the American Heart Association. Circulation 2019;139(10):e56-e528. doi: 10.1161/CIR.0000000000000659.
3. Roth GA, Johnson C, Abajobir A, Abd-Allah F, Abera SF, Abyu G, Ahmed M, Aksut B, Alam T, Alam K, et al. Global, Regional, and National Burden of Cardiovascular Diseases for 10 Causes, 1990 to 2015. Edtion ed. J Am Coll Cardiol, 2017:1-25.
4. Torio CM, Moore BJ. National Inpatient Hospital Costs: The Most Expensive Conditions by Payer, 2013: Statistical Brief #204. Edtion ed. Healthcare Cost and Utilization Project (HCUP) Statistical Briefs. Rockville (MD): Agency for Healthcare Research and Quality (US), 2006.
5. Kim MK, Shin J, Kweon SS, Shin DH, Lee YH, Chun BY, Choi BY. Harmful and beneficial relationships between alcohol consumption and subclinical atherosclerosis. Nutr Metab Cardiovasc Dis 2014;24(7):767-76. doi: 10.1016/j.numecd.2014.02.004 10.1016/j.numecd.2014.02.004. Epub 2014 Feb 15.
6. Fernández-Solà J. Cardiovascular risks and benefits of moderate and heavy alcohol consumption. Nature Reviews Cardiology 2015;12:576-87. doi: 10.1038/nrcardio.2015.91.
7. Zhang C, Qin Y-Y, Chen Q, Jiang H, Chen X-Z, Xu C-L, Mao P-J, He J, Zhou Y-H. Alcohol intake and risk of stroke: A dose–response meta-analysis of prospective studies. International Journal of Cardiology 2014;174:669-77. doi: 10.1016/j.ijcard.2014.04.225.
8. Zheng Y-L, Lian F, Shi Q, Zhang C, Chen Y-W, Zhou Y-H, He J. Alcohol intake and associated risk of major cardiovascular outcomes in women compared with men: a systematic review and meta-analysis of prospective observational studies. BMC Public Health 2015;15:773. doi: 10.1186/s12889-015-2081-y.
9. Ricci C, Wood A, Muller D, Gunter MJ, Agudo A, Boeing H, van der Schouw YT, Warnakula S, Saieva C, Spijkerman A, et al. Alcohol intake in relation to non-fatal and fatal coronary heart disease and stroke: EPIC-CVD case-cohort study. BMJ (Clinical research ed) 2018;361:k934. doi: 10.1136/bmj.k934.
10. Castelli WP, Doyle JT, Gordon T, Hames CG, Hjortland MC, Hulley SB, Kagan A, Zukel WJ. HDL cholesterol and other lipids in coronary heart disease. The cooperative lipoprotein phenotyping study. Circulation 1977;55(5):767-72. doi: 10.1161/01.cir.55.5.767 10.1161/01.cir.55.5.767.
11. Miller NE, Thelle DS, Forde OH, Mjos OD. The Tromso heart-study. High-density lipoprotein and coronary heart-disease: a prospective case-control study. Lancet (London, England) 1977;1(8019):965-8. doi: 10.1016/s0140-6736(77)92274-7 10.1016/s0140-6736(77)92274-7.
12. Goff DC, Jr., Lloyd-Jones DM, Bennett G, Coady S, D'Agostino RB, Gibbons R, Greenland P, Lackland DT, Levy D, O'Donnell CJ, et al. 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation 2013;129(25 Suppl 2):S49-73. doi: 10.1161/01.cir.0000437741.48606.98 10.1161/01.cir.0000437741.48606.98. Epub 2013 Nov 12.

13. Toth PP, Barter PJ, Rosenson RS, Boden WE, Chapman MJ, Cuchel M, D'Agostino RB, Sr., Davidson MH, Davidson WS, Heinecke JW, et al. High-density lipoproteins: a consensus statement from the National Lipid Association. J Clin Lipidol 2013;7(5):484-525. doi: 10.1016/j.jacl.2013.08.001
10.1016/j.jacl.2013.08.001. Epub 2013 Aug 11.
14. Rimm EB, Moats C. Alcohol and Coronary Heart Disease: Drinking Patterns and Mediators of Effect. Annals of Epidemiology 2007;17:S3-S7. doi: 10.1016/j.annepidem.2007.01.002.
15. Magnus P, Bakke E, Hoff DA, Høiseth G, Graff-Iversen S, Knudsen GP, Myhre R, Normann PT, Næss Ø, Tambs K, et al. Controlling for high-density lipoprotein cholesterol does not affect the magnitude of the relationship between alcohol and coronary heart disease. Circulation 2011;124:2296-302. doi: 10.1161/CIRCULATIONAHA.111.036491.
16. Rader DJ, Hovingh GK. HDL and cardiovascular disease. The Lancet 2014;384:618-25. doi: 10.1016/S0140-6736(14)61217-4.
17. Jin C, Chen S, Vaidya A, Wu Y, Wu Z, Hu FB, Kris-Etherton P, Wu S, Gao X. Longitudinal Change in Fasting Blood Glucose and Myocardial Infarction Risk in a Population Without Diabetes. Diabetes Care 2017;40(11):1565-72. doi: 10.2337/dc17-0610.
18. Huang S, Li J, Shearer GC, Lichtenstein AH, Zheng X, Wu Y, Jin C, Wu S, Gao X. Longitudinal study of alcohol consumption and HDL concentrations: a community-based study. The American journal of clinical nutrition 2017;105:905-12. doi: 10.3945/ajcn.116.144832.
19. Bild DE, Bluemke DA, Burke GL, Detrano R, Diez Roux AV, Folsom AR, Greenland P, Jacob DR, Kronmal R, Liu K, et al. Multi-Ethnic Study of Atherosclerosis: objectives and design. American journal of epidemiology 2002;156:871-81.

Chapter 2
Review of the literature

Alcohol intake, high density lipoprotein and MI: a review from epidemiological studies

This section of the dissertation is a review of the literature on the associations between 1) alcohol intake and risk of MI, 2) alcohol intake and high density lipoprotein (HDL), 3) HDL and risk of cardiovascular disease among the general population, and 4) alcohol intake, HDL, and MI.

Introduction

Myocardial infarction (MI) is the most common form of coronary heart disease, which is by far the commonest cardiovascular condition. Despite the improvement and increased use in evidence-based therapies and lifestyle interventions (1, 2), it remains the number one cause of morbidity and mortality worldwide. In 2015, it's estimated that there were 7.29 million acute MIs worldwide (3). In the US, the estimated annual incidence of MI is 805,000 including 605,000 new heart attacks and 200,000 recurrent heart attacks (2) and it causes more than 111,777 deaths. The estimated cost of MI is tremendous; in 2013, MI was the most expensive condition treated in US hospitals with hospital costs of US $12.1 billion (4). Therefore, it is important to identify and examine modifiable risk factors for disease control and prevention.

Unlike other causes for ischemic heart disease, such as unstable angina, MI occurs when there is cell death, as measured by a blood test of biomarkers, i.e. the cardiac protein troponin or the cardiac enzyme CK-MB. The myocardial cell death is due to prolonged ischemia, which

mostly results from a disruption of a vulnerable atherosclerotic plaque or erosion of the coronary artery endothelium (5). Formation of such atherosclerotic plaque or erosion is related to a variety of risk factors. Known risk factors for MI include age, sex, race and ethnicity, inadequate physical activity, unhealthy diet, family history of MI, metabolic syndrome (i.e. increased waist circumference, elevated triglycerides, elevated blood pressure, elevated glucose, and low HDL-C), hypercholesterolemia, chronic kidney disease, and chronic inflammatory conditions (6).

Alcohol intake is one risk factor that has both a beneficial and a harmful influence on atherosclerosis, specifically MI. On one hand, high-dose alcohol consumption induces arterial damage in coronary, brain and peripheral limb arteries, and increases individual cardiovascular risk factors such as hypertension, hypertriglyceridemia, which are thought to be responsible for the increased cardiovascular risk (7, 8). On the other hand, light-to-moderate alcohol consumption is generally thought to reduce the risk of cardiovascular disease, particularly atherosclerosis-induced disease including coronary disease and ischemic stroke, through elevated HDL-C levels, accelerated glucose metabolism, and anti-inflammatory effects (9-11).

Being one of the most noticed predictors for MI, HDL has been proposed to be a mediator on the pathway between alcohol intake and risk of MI (12). However, the causal relation between HDL and atherosclerosis remains uncertain. Controversial results have been generated from epidemiological studies, human genetics and failed clinical trials and thus have raised skepticism about the role of HDL in protecting against cardiovascular disease (13).

Alcohol intake and risk of MI

The relationship between alcohol intake and cardiovascular disease has attracted significant interest in epidemiological studies. As for coronary heart disease, alcohol was described to afford relief for angina pectoris (14) by Heberden in 1786, and was thought to be a coronary vasodilator. However later findings showed that alcohol does not improve myocardial oxygen deficiency, and does not immediately affect coronary blood flow (15-17). Because angina pectoris is a subjective symptom and is difficult to quantify, it is little used in epidemiological studies.

Inverse association between light-to-moderate alcohol and MI

The inverse association between alcohol intake and atherosclerotic disease was first reported in 1904 (18). Since then, hundreds of observational case-control or cohort studies have shown that light-to-moderate alcohol intake (i.e. 10-30g per day) was related to a lower risk of coronary heart disease, including MI (10, 11, 19-27). One early meta-analysis regarding MI including 12 prospective cohorts and two case-control studies from 1978 to 1998 found that a relative small dose (i.e. 1-4 drinks a day) of wine, beer or spirits are associated with a slightly reduced risk for MI but not high doses of alcohol (i.e. 5 drinks a day), compared to nondrinkers (the authors did not assess lifelong abstainers) (28). Populations involved in this study included cohorts from Finland, Japan, American Framingham, and Yugoslavia. Similarly, the lowest risk of MI was related to a small dose of an average 3 drinks/wk (1 standard drink = 14g ethanol) in the Costa Rican population, relative to lifelong abstainers (29). In the INTERHEART study involving 52 countries worldwide, any alcohol use was related to a lower risk for MI regardless of sex and age, 1-4 times per week was related to the lowest risk of MI (HR=0.839, 95%CI: 0.751-0.937). However, an increased risk of MI among South Asian alcohol consumers has been observed (30). In contrast to the consistent lower risk observed for light-to-moderate amounts,

some studies reported a continuous decreasing risk of MI related to increasing alcohol intake. In the Copenhagen City Heart Study, increased alcohol intake as much as to 14+ drinks/week for women and 21+ drinks/week for men (relative to <1 drink/week, 1 drink=12g ethanol) was related to decreasing risk of MI among both men and women, and such association was not modified by alcohol dehydrogenase genotype ADH1C (31). In a most recent combined analysis of individuals from 83 prospective cohort studies, the researchers again found an inverse log-linear association between alcohol intake and risk of MI (32).

Regarding to the validity of such association, some methodological issues have been raised. One of them is that some analyses grouped lifelong abstainers and ex-drinkers together and therefore a false impression of benefit from light-to-moderate drinking occurred if the ex-drinkers who were "sick quitters" with increased CHD risk were included in the nondrinking referent group. Another methodological issue is the adjustment of baseline CHD. However, cohort studies separating ex-drinkers and lifelong abstainers as two categories and controlling for baseline CHD refuted such issues (20, 27, 32).

Different alcoholic beverages and risk of MI

The French Paradox suggests that red wine is superior to other alcoholic beverages when it comes to cardiovascular benefits (33). Some earlier ecological studies found that moderate consumption of wine rich in polyphenols (34) including resveratrol and flavonoids could confer more cardiovascular protection than beer and spirits (19). Of note, results from ecological studies sometimes can be faulty due to lack of individual data and inadequately controlling for major confounders. In a Northern California cohort of 128, 934 adults, wine was found to have a lower risk of mortality than beer or spirits drinkers, but the authors concluded that it is unclear whether the reduced risk is due to the nonalcoholic wine ingredients, drinking pattern or associated traits (35). In some other studies, wine and beer were found to be equally beneficial but greater than liquor. In the same Northern California cohort as mentioned above, Klatsky et al. found that

before adjustment of total alcohol, wine, beer and liquor all showed evidence for coronary protection but after adjustment of total alcohol, only beer and wine remained statistically significant in relation to a lower risk for CHD hospitalization (36). Similarly, two closely overlapped J-shaped curves modeling vascular risk (including both fatal and nonfatal cardiovascular disease (CVD)) relation to wine and beer were observed, with a maximal protection of 33% at 25g/day of alcohol by either beverage (37).

Nevertheless, evidence has also shown that one type of drink was not more cardioprotective than the others. Significant inverse association between risk of heart disease and moderate drinking was observed for wine, beer and spirits in relation to both cohort studies and case-control studies (19, 38). After fully controlling age, sex, smoke, BMI and other potential confounders, similar RR for MI was oberverd for each type of alcoholic beverage (1.09 for beer, 0.97 for wine, and 0.83 for beer) in 680 cases and controls from the Boston Area Health Study (38). Systematic reviews found that doses of 1-4 drinks a day of wine, beer, and spirits are euqally beneficial for MI (a relative risk of 0.8, estimated from the figure provided in the paper) (28).

Therefore, there seems to not be a clear answer as to which type of alcoholic beverage is more cardioprotective. Beverage choice could be complicated by probable confounding such as the fact that wine drinkers in France or other main wine consumption countries have the most favorable heart disease risk profile (39). Usual pattern of wine drinking may also be important because it is more often consumed slowly with meals (40) or snacks. Polyphenols in wine or beer seems to have antioxidant (41), anti-inflammatory (42), or hypotensive (43) effects. However, within the moderate alcohol consumption context, the concentrations of the polyphenolic metabolites that reach the human are very low and such potential effects may not always be achieved in human clinical trials. Thus, it is unlikely that the antioxidant, anti-inflammatory or hypotensive effect of alcohol are the primary mechanism for the protection against CHD.

Therefore, the ethyl alcohol is probably the main factor that confers the benefit for heart disease (8).

Drinking pattern and risk of MI

Alcohol drinking pattern is an important factor in explaining the effects of alcohol upon cardiovascular heath in populations (21, 29, 30, 44-49). Binge drinking or irregular heavy drinking has been consistently observed to have a harmful effect on MI, CHD or total mortality. One meta-analysis including 4 cohorts and 2 case-control studies found that compared with the abstainers, heavy irregular or binge drinkers had a higher CHD risk (RR=1.1, 95%CI: 0.64, 0.89), whereas regular heavy drinkers had an RR of 0.75 (95% CI: 0.64, 0.89) (21). In a hospital-based case-control study in Serbia, habitual binge drinking was also found to relate to higher odds for MI (OR=2.2, 95%CI: 1.2, 4.2)(50). Both chronic and episodic heavy drinking does not confer any beneficial effect on ischemic heart disease (IHD); alcohol use disorder has a 1.5- to 2- fold higher risk for IHD (48).

Another factor of interest is variability of drinking pattern over time. There is study showing that moderate drinkers who continue to consume alcohol after an acute MI (AMI) had a high physical functioning although one possible explanation was that the quitters are secondary to a significant lifestyle change or being too sick. However, after the authors adjusted for the co-morbidities and other potential sources of confounding, there was no evidence observed for harm by quitting alcohol in moderate after an AMI (51). Interestingly, in a study where King et al. followed middle-aged nondrinkers and compared participants who began to drink to those who continued to abstain, the cohort that began to drink had a 38% lower risk of developing cardiovascular disease than the cohort who continued to abstain from alcohol (52).

HDL and cardiovascular risk

A short history about the HDL hypothesis

According to Kingwell et al (53), the discovery of HDL dates back to 1929 when a protein-rich, lipid-poor complex was isolated from equine serum. The advancement in ultracentrifuge techniques made it possible to isolate HDL in humans in the 1950s (54) and later on to measure the cholesterol content of HDL. Such improvement in techniques enabled large-scale epidemiological studies between HDL cholesterol (HDL-C) concentrations and risk of CHD. As early as 1951 (55), the observation was made that healthy men had higher levels of alpha (i.e. high density) lipoprotein, measured as fraction of total cholesterol, than did men with CHD. Afterwards, more observational studies reported a deficiency of HDL in plasma was a risk factor for IHD (56). Such findings formed a basis for the concept that HDL-C as a good cholesterol may have properties that protect against CHD and therefore interventions to increase HDL may reduce the risk for CHD. Glomset and colleagues (57) identified the role of plasma lecithin: cholesterol acyltransferase (LCAT) and originated the concept of reverse cholesterol transport of HDL. The hypothesis was advanced in 1970s, when Miller et al. proposed that "a reduction of plasma HDL concentration may accelerate the development of atherosclerosis, and hence IHD, by impairing the clearance of cholesterol from the arterial wall" (58).

The hypothesis that HDL protects against IHD has been further supported subsequently by both animal studies and epidemiological studies. Infusion of HDL into rabbits was found to inhibit atherosclerosis by Badimon et al (59). Transgenic mice with high plasma apoA-I and HDL levels were significantly protected from the development of preatherosclerotic fatty lesions (60), and overexpression of apoA-1 in viral injected mice with pre-existing atherosclerosis were also found to regress the atherosclerotic disease (61). Large prospective cohort data from different

racial and ethnic groups worldwide has also confirmed that HDL-C is independently related to a lower risk of incident MI and stroke (62-65).

Challenges to the HDL hypothesis

However, the HDL hypothesis has been challenged by data from human genetic studies and randomized controlled trials. The three main genetic disorders causing extreme low HDL-C include mutations in apoA-I, ABCA1, and LCAT. Even though the HDL concentrations in those studies are less than the 5th percentile of the population, none of the disorders is unequivocally associated with premature coronary heart disease (66). On the other hand, mutations that elevated HDL-C levels, such as mutations causing cholesteryl ester transfer protein (CETP) deficiency, or affecting hepatic lipase and scavenger receptor class B member 1 (SRB1) did not provide a clear picture of the link between HDL metabolism and atherosclerosis. In one meta-analysis, a mutation of B2 allele of the Taq1B SNP (which is associated with low CETP activity) (67) and elevated HDL-C was related to a lower risk of CHD, but such association was found to be limited to a Chinese population in another meta-analysis (68). Thus, it is still not clear whether CETP deficiency protects against CHD (13).

Findings from clinical trials using drugs targeted to increase HDL-C have also been controversial. Two recent trials (AIM-HIGH (69) and HPS2-THRIVE (70)) giving niacin to patients who have been using statin therapy failed to reduce cardiovascular events although HDL-C was increased. Therefore, extended-release niacin added to a statin in patients with reasonably controlled LDL-C concentrations does not have a further cardiovascular benefit despite a slight increase in HDL-C concentrations (13). Fibrates have potential effects on increasing lipoprotein lipase activity, increasing synthesis of apoA-I and apoA-2, and possibly increasing expression of ABCA1 transporters (53). Although in the Helsinki Heart Study (71) and VA-HIT Study (72), fibrates have been shown to increase HDL-C and further prevent CHD events, the other two trials

including BIP trial and ACCORD study did not find a significant effect of bezafibrate on secondary CHD events nor an effect of fenofibrate added to a statin on CVD in patients with type 2 diabetes. Trials with dalcetrapib, and evacetrapib, a class of drugs that elevate the concentration of large, mature HDL by inhibiting cholesteryl ester transfer protein (CETP) were terminated prematurely due to lack of efficacy (70, 73, 74). Taken together, these data suggest that HDL cholesterol is not causally atheroprotective.

Alternative choices

HDL is a complicated particle comprised of heterogeneous particles with different functional properties including cholesterol, cholesterol esters, phospholipids, triglycerides, and apolipoproteins. HDL-C, which is used as a biomarker for assessing risk of CVD, represents the cholesterol content of HDL. Emerging evidence has shown that HDL-C does not capture all the cardiovascular protective effect of HDL particles. For example, Khera et al. (75). found that the capacity of HDL to promote cholesterol efflux from macrophages is related to a lower risk of coronary disease independent of HDL-C level. The main component of HDL, apolipoprotein A-1 (apo A-1), is largely responsible for reverse cholesterol transport through the macrophage ATP-binding cassette transporter (ABCA1). Small peptides that mimic some of the properties of apoA-1 have been shown in preclinical models to improve HDL function and reduce atherosclerosis without altering HDL-cholesterol levels (76). Using the Multi-Ethnic Study of Atherosclerosis data, Mackey et al. found that per 15mg/dl higher HDL-C was associated with 26% lower risk of coronary heart risk, but the association was no longer observed after adjusting for HDL particles, defined as the sum of the particle concentrations of HDL subclasses, which are quantified based on particle size using the amplitudes of its lipid methyl group nuclear magnetic resonance (NMR) signals (77). Should the alternative function indicators be better therapeutic target of HDL remains to be examined?

Alcohol intake, HDL and cardiovascular risk

Alcohol intake and HDL

The effect of alcohol intake on HDL was first reported from the cooperative lipoprotein phenotyping study. Alcohol consumption was found to be positively associated with HDL cholesterol in a combined population from Albany, Evans County, Framingham, Honolulu, and San Francisco Studies (78). Alcohol consumption may have an effect on both HDL levels and HDL subclasses. Alcohol is reported to raise plasma levels of both total HDL and its subfractions including both HDL_2 and HDL_3 (79, 80) as well as major HDL lipoproteins, including apolipoprotein A-I and apo A–Π (80). Alcohol may also increase both the total phospholipid (PL) and polyunsaturated fatty acid content of HDL (81), which may increase the fluidity of HDL. Further, alcohol promotes the conversion of phosphatidylcholine to phosphstidylethanol in HDL, which increases HDL binding to endothelial cells and up-regulates vascular endothelial growth factor, protecting against atherosclerosis (82).

Cross-sectional studies have found a linear association between alcohol intake and HDL concentration (83-86). Few longitudinal studies (87-89) have assessed alcohol consumption and lipids at least twice, but they investigated the relationships of a time-dependent change in alcohol consumption with the change in HDL, and found that an increase in alcohol intake was positively associated with an increase in HDL. Clinical trials have also assessed the effect of alcohol intake on HDL, but most have not been able to investigate the dose-response relationship and also was limited by short term and small sample size (80, 90). Two meta-analyses (80, 90) have summarized the effect of alcohol consumption on biological markers associated with

cardiovascular disease risk from interventional studies, and reported a dose-response between alcohol consumption and HDL cholesterol: HDL increased linearly as alcohol intake increased.

Alcohol intake, HDL and MI

The observed J-shaped association between alcohol intake and CVD has been proposed to be mediated through HDL-C. Several cohort studies (38, 88, 91-94) examining the interrelationship among alcohol use, HDL level and myocardial infarction (MI) risk and/or mortality of coronary heart disease have generated controversial results. In most of the studies, including the Lipid Research Clinics Follow-up Study, the Honolulu Heart Program, Multiple Risk Factor Intervention Trial, and the Health Professional follow-up, higher HDL-C concentration could explain approximately 50% of the CVD preventive effects from alcoholic beverages. However, in the Norway Study cohort, Magnus et al. found a very slight change in the hazard ratio of alcohol intake after including HDL-C in the Cox regression model, and concluded that HDL-C is not an important variable in the possible pathway between moderate alcohol intake and CHD benefits (95). These disparate findings raised the questions whether alcohol falls into the class of HDL-raisers that does not only increase HDL-C but also further reduce CVD risk, or the class that increases HDL-C but does not reduce CVD risk due to increased HDL, and whether the impact of alcohol intake on CVD be mediated more by HDL indices reflecting HDL functional properties or metabolism, such as HDL particles or particle size, or apolipoproteins, relative to HDL-C.

References

1. Reed GW, Rossi JE, Cannon CP. Acute myocardial infarction. Lancet (London, England) 2017;389(10065):197-210. doi: 10.1016/s0140-6736(16)30677-8.
2. Benjamin EJ, Muntner P, Alonso A, Bittencourt MS, Callaway CW, Carson AP, Chamberlain AM, Chang AR, Cheng S, Das SR, et al. Heart Disease and Stroke Statistics-2019 Update: A Report From the American Heart Association. Circulation 2019;139(10):e56-e528. doi: 10.1161/CIR.0000000000000659.
3. Roth GA, Johnson C, Abajobir A, Abd-Allah F, Abera SF, Abyu G, Ahmed M, Aksut B, Alam T, Alam K, et al. Global, Regional, and National Burden of Cardiovascular Diseases for 10 Causes, 1990 to 2015. Edtion ed. J Am Coll Cardiol, 2017:1-25.
4. Torio CM, Moore BJ. National Inpatient Hospital Costs: The Most Expensive Conditions by Payer, 2013: Statistical Brief #204. Edtion ed. Healthcare Cost and Utilization Project (HCUP) Statistical Briefs. Rockville (MD): Agency for Healthcare Research and Quality (US), 2006.
5. Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA, White HD, Executive Group on behalf of the Joint European Society of Cardiology /American College of Cardiology /American Heart Association /World Heart Federation Task Force for the Universal Definition of Myocardial I. Fourth Universal Definition of Myocardial Infarction (2018). J Am Coll Cardiol 2018;72(18):2231-64. doi: 10.1016/j.jacc.2018.08.1038.
6. Arnett DK, Blumenthal RS, Albert MA, Buroker AB, Goldberger ZD, Hahn EJ, Himmelfarb CD, Khera A, Lloyd-Jones D, McEvoy JW, et al. 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease. Circulation 2019:Cir0000000000000678. doi: 10.1161/cir.0000000000000678 10.1161/CIR.0000000000000678.
7. Kim MK, Shin J, Kweon SS, Shin DH, Lee YH, Chun BY, Choi BY. Harmful and beneficial relationships between alcohol consumption and subclinical atherosclerosis. Nutr Metab Cardiovasc Dis 2014;24(7):767-76. doi: 10.1016/j.numecd.2014.02.004 10.1016/j.numecd.2014.02.004. Epub 2014 Feb 15.
8. Fernández-Solà J. Cardiovascular risks and benefits of moderate and heavy alcohol consumption. Nature Reviews Cardiology 2015;12:576-87. doi: 10.1038/nrcardio.2015.91.
9. Zhang C, Qin Y-Y, Chen Q, Jiang H, Chen X-Z, Xu C-L, Mao P-J, He J, Zhou Y-H. Alcohol intake and risk of stroke: A dose–response meta-analysis of prospective studies. International Journal of Cardiology 2014;174:669-77. doi: 10.1016/j.ijcard.2014.04.225.
10. Zheng Y-L, Lian F, Shi Q, Zhang C, Chen Y-W, Zhou Y-H, He J. Alcohol intake and associated risk of major cardiovascular outcomes in women compared with men: a systematic review and meta-analysis of prospective observational studies. BMC Public Health 2015;15:773. doi: 10.1186/s12889-015-2081-y.
11. Ricci C, Wood A, Muller D, Gunter MJ, Agudo A, Boeing H, van der Schouw YT, Warnakula S, Saieva C, Spijkerman A, et al. Alcohol intake in relation to non-fatal and fatal coronary heart disease and stroke: EPIC-CVD case-cohort study. BMJ (Clinical research ed) 2018;361:k934. doi: 10.1136/bmj.k934.
12. Ronksley PE, Brien SE, Turner BJ, Mukamal KJ, Ghali Wa. Association of alcohol consumption with selected cardiovascular disease outcomes: a systematic review and meta-analysis. Bmj 2011;342:d671-d. doi: 10.1136/bmj.d671.

13. Rader DJ, Hovingh GK. HDL and cardiovascular disease. The Lancet 2014;384:618-25. doi: 10.1016/S0140-6736(14)61217-4.
14. Klatsky AL. Alcohol and cardiovascular diseases: a historical overview. Ann N Y Acad Sci 2002;957:7-15. doi: 10.1111/j.1749-6632.2002.tb02901.x.
15. Russek HI, Naegele CF, Regan FD. Alcohol in the treatment of angina pectoris. J Am Med Assoc 1950;143(4):355-7. doi: 10.1001/jama.1950.02910390027008
10.1001/jama.1950.02910390027008.
16. Orlando J, Aronow WS, Cassidy J, Prakash P. Effect of ethanol on angina pectoris. Ann Intern Med 1976;84(6):652-5. doi: 10.7326/0003-4819-84-6-652
10.7326/0003-4819-84-6-652.
17. Klatsky AL. Epidemiology of coronary heart disease--influence of alcohol. Alcohol Clin Exp Res 1994;18(1):88-96.
18. Cabot RC. THE RELATION TO ALCOHOL TO ARTERIOSCLEROSIS. Journal of the American Medical Association 1904;XLIII(12):774-5. doi: 10.1001/jama.1904.92500120002a.
19. Rimm EB, Klatsky a, Grobbee D, Stampfer MJ. Review of moderate alcohol consumption and reduced risk of coronary heart disease: is the effect due to beer, wine, or spirits. BMJ (Clinical research ed) 1996;312:731-6. doi: 10.1136/bmj.312.7033.731.
20. Corrao G, Rubbiati L, Bagnardi V, Zambon A, Poikolainen K. Alcohol and coronary heart disease: a meta-analysis. Addiction (Abingdon, England) 2000;95:1505-23.
21. Bagnardi V, Zatonski W, Scotti L, La Vecchia C, Corrao G. Does drinking pattern modify the effect of alcohol on the risk of coronary heart disease? Evidence from a meta-analysis. Journal of Epidemiology & Community Health 2008;62:615-9. doi: 10.1136/jech.2007.065607.
22. Friedman LA, Kimball AW. Coronary heart disease mortality and alcohol consumption in Framingham. AmJEpidemiol 1986;124:481-9.
23. Bazzano LA, Gu D, Reynolds K, Chen J, Wu X, Chen C-S, Duan X, Chen J, He J. Alcohol consumption and risk of coronary heart disease among Chinese men. International Journal of Cardiology 2009;135:78-85. doi: 10.1016/j.ijcard.2008.03.038.
24. Ikehara S, Iso H, Yamagishi K, Yamamoto S, Inoue M, Tsugane S, Group JS. Alcohol Consumption, Social Support, and Risk of Stroke and Coronary Heart Disease Among Japanese Men: The JPHC Study. Alcoholism: Clinical and Experimental Research 2009;33:1025-32. doi: 10.1111/j.1530-0277.2009.00923.x.
25. Corrao G, Bagnardi V, Zambon A, La Vecchia C. A meta-analysis of alcohol consumption and the risk of 15 diseases. Preventive Medicine 2004;38:613-9. doi: 10.1016/j.ypmed.2003.11.027.
26. Costanzo S, Di Castelnuovo A, Donati MB, Iacoviello L, De Gaetano G. Wine, beer or spirit drinking in relation to fatal and non-fatal cardiovascular events: A meta-analysis. European Journal of Epidemiology, 2011:833-50.
27. Ronksley PE, Brien SE, Turner BJ, Mukamal KJ, Ghali WA. Association of alcohol consumption with selected cardiovascular disease outcomes: a systematic review and meta-analysis. BMJ (Clinical research ed) 2011;342:d671. doi: 10.1136/BMJ.D671.
28. Cleophas TJ. Wine, beer and spirits and the risk of myocardial infarction: A systematic review. Biomedicine and Pharmacotherapy 1999;53:417-23. doi: 10.1016/S0753-3322(99)80121-8.
29. Kabagambe EK, Baylin A, Ruiz-Narvaez E, Rimm EB, Campos H. Alcohol intake, drinking patterns, and risk of nonfatal acute myocardial infarction in Costa Rica. Am J Clin Nutr 2005;82(6):1336-45. doi: 10.1093/ajcn/82.6.1336.

30. Leong DP, Smyth A, Teo KK, McKee M, Rangarajan S, Pais P, Liu L, Anand SS, Yusuf S. Patterns of alcohol consumption and myocardial infarction risk: Observations from 52 countries in the INTERHEART case-control study. Circulation 2014;130:390-8. doi: 10.1161/CIRCULATIONAHA.113.007627.
31. Tolstrup JS, Gronbaek M, Nordestgaard BG. Alcohol intake, myocardial infarction, biochemical risk factors, and alcohol dehydrogenase genotypes. Circ Cardiovasc Genet 2009;2(5):507-14. doi: 10.1161/CIRCGENETICS.109.873604.
32. Wood AM, Kaptoge S, Butterworth AS, Willeit P, Warnakula S, Bolton T, Paige E, Paul DS, Sweeting M, Burgess S, et al. Risk thresholds for alcohol consumption: combined analysis of individual-participant data for 599 912 current drinkers in 83 prospective studies. The Lancet 2018;391:1513-23. doi: 10.1016/S0140-6736(18)30134-X.
33. Renaud S, Gueguen R. The French paradox and wine drinking. Novartis Found Symp 1999;216:208-17; discussion 17-22, 152-8.
34. Wu JM, Hsieh TC. Resveratrol: a cardioprotective substance. Ann N Y Acad Sci 2011;1215:16-21. doi: 10.1111/j.1749-6632.2010.05854.x
10.1111/j.1749-6632.2010.05854.x.
35. Klatsky AL, Friedman GD, Armstrong MA, Kipp H. Wine, liquor, beer, and mortality. Am J Epidemiol 2003;158(6):585-95. doi: 10.1093/aje/kwg184
10.1093/aje/kwg184.
36. Klatsky AL, Armstrong MA, Friedman GD. Red wine, white wine, liquor, beer, and risk for coronary artery disease hospitalization. Am J Cardiol 1997;80(4):416-20. doi: 10.1016/s0002-9149(97)00388-3
10.1016/s0002-9149(97)00388-3.
37. Costanzo S, Di Castelnuovo A, Donati MB, Iacoviello L, de Gaetano G. Wine, beer or spirit drinking in relation to fatal and non-fatal cardiovascular events: a meta-analysis. Eur J Epidemiol 2011;26(11):833-50. doi: 10.1007/s10654-011-9631-0
10.1007/s10654-011-9631-0. Epub 2011 Nov 11.
38. Gaziano JM, Hennekens CH, Godfried SL, Sesso HD, Glynn RJ, Breslow JL, Buring JE. Type of alcoholic beverage and risk of myocardial infarction. The American journal of cardiology 1999;83:52-7. doi: S0002-9149(98)00782-6 [pii].
39. Klatsky AL, Armstrong MA, Kipp H. Correlates of alcoholic beverage preference: traits of persons who choose wine, liquor or beer. Br J Addict 1990;85(10):1279-89.
40. Klatsky AL. Alcohol and cardiovascular diseases: where do we stand today? J Intern Med 2015;278(3):238-50. doi: 10.1111/joim.12390.
41. Vinson JA, Mandarano M, Hirst M, Trevithick JR, Bose P. Phenol antioxidant quantity and quality in foods: beers and the effect of two types of beer on an animal model of atherosclerosis. J Agric Food Chem 2003;51(18):5528-33. doi: 10.1021/jf034189k
10.1021/jf034189k.
42. Palmieri D, Pane B, Barisione C, Spinella G, Garibaldi S, Ghigliotti G, Brunelli C, Fulcheri E, Palombo D. Resveratrol counteracts systemic and local inflammation involved in early abdominal aortic aneurysm development. J Surg Res 2011;171(2):e237-46. doi: 10.1016/j.jss.2011.07.041
10.1016/j.jss.2011.07.041. Epub 2011 Aug 24.
43. Bhatt SR, Lokhandwala MF, Banday AA. Resveratrol prevents endothelial nitric oxide synthase uncoupling and attenuates development of hypertension in spontaneously hypertensive rats. Eur J Pharmacol 2011;667(1-3):258-64. doi: 10.1016/j.ejphar.2011.05.026
10.1016/j.ejphar.2011.05.026. Epub 2011 May 27.

44. Tolstrup J, Jensen MK, Tjonneland A, Overvad K, Mukamal KJ, Gronbaek M. Prospective study of alcohol drinking patterns and coronary heart disease in women and men. Bmj 2006;332(7552):1244-8. doi: 10.1136/bmj.38831.503113.7C
10.1136/bmj.38831.503113.7C. Epub 2006 May 3.
45. Rimm EB, Moats C. Alcohol and Coronary Heart Disease: Drinking Patterns and Mediators of Effect. Annals of Epidemiology 2007;17:S3-S7. doi: 10.1016/j.annepidem.2007.01.002.
46. Galan I, Valencia-Martin JL, Guallar-Castillon P, Rodriguez-Artalejo F. Alcohol drinking patterns and biomarkers of coronary risk in the Spanish population. Nutr Metab Cardiovasc Dis 2014;24(2):189-97. doi: 10.1016/j.numecd.2013.07.002
10.1016/j.numecd.2013.07.002. Epub 2014 Jan 10.
47. Bobak M, Malyutina S, Horvat P, Pajak A, Tamosiunas A, Kubinova R, Simonova G, Topor-Madry R, Peasey A, Pikhart H, et al. Alcohol, drinking pattern and all-cause, cardiovascular and alcohol-related mortality in Eastern Europe. Eur J Epidemiol 2015;31(1):21-30. doi: 10.1007/s10654-015-0092-8
10.1007/s10654-015-0092-8. Epub 2015 Oct 14.
48. Roerecke M, Rehm J. Alcohol consumption, drinking patterns, and ischemic heart disease: a narrative review of meta-analyses and a systematic review and meta-analysis of the impact of heavy drinking occasions on risk for moderate drinkers. BMC Med 2015;12:182. doi: 10.1186/s12916-014-0182-6
10.1186/s12916-014-0182-6.
49. Zhang Y, Yu Y, Yuan Y, Yu K, Yang H, Li X, Min X, Zhang C, He M, Zhang X, et al. Association of drinking pattern with risk of coronary heart disease incidence in the middle-aged and older Chinese men: Results from the Dongfeng-Tongji cohort. PloS one 2017;12(5):e0178070. doi: 10.1371/journal.pone.0178070
10.1371/journal.pone.0178070. eCollection 2017.
50. Ilic M, Grujicic Sipetic S, Ristic B, Ilic I. Myocardial infarction and alcohol consumption: A case-control study. Edtion ed. PloS one, 2018.
51. Carter MD, Lee JH, Buchanan DM, Peterson ED, Tang F, Reid KJ, Spertus JA, Valtos J, O'Keefe JH. Comparison of outcomes among moderate alcohol drinkers before acute myocardial infarction to effect of continued versus discontinuing alcohol intake after the infarct. Am J Cardiol 2010;105(12):1651-4. doi: 10.1016/j.amjcard.2010.01.339.
52. King DE, Mainous AG, 3rd, Geesey ME. Adopting moderate alcohol consumption in middle age: subsequent cardiovascular events. Am J Med 2008;121(3):201-6. doi: 10.1016/j.amjmed.2007.12.004
10.1016/j.amjmed.2007.12.004.
53. Miller BAKMJCAKaNE. HDL-targeted therapies: progress, failures and future. Nature Review 2014;13:445.
54. Russ EM, Eder HA, Barr DP. Protein-lipid relationships in human plasma. I. In normal individuals. Am J Med 1951;11(4):468-79. doi: 10.1016/0002-9343(51)90182-9
10.1016/0002-9343(51)90182-9.
55. Barr DP, Russ EM, Eder HA. Protein-lipid relationships in human plasma. II. In atherosclerosis and related conditions. Am J Med 1951;11(4):480-93. doi: 10.1016/0002-9343(51)90183-0
10.1016/0002-9343(51)90183-0.
56. Gofman JW, Malamud N, Simon A, Waters ES, Young W. The interrelationship between cerebral and coronary atherosclerosis; a preliminary report. Geriatrics 1956;11(9):413-8.

57. Glomset JA, Janssen ET, Kennedy R, Dobbins J. Role of plasma lecithin:cholesterol acyltransferase in the metabolism of high density lipoproteins. J Lipid Res 1966;7(5):638-48.
58. Miller GJ, Miller NE. Plasma-high-density-lipoprotein concentration and development of ischaemic heart-disease. Lancet (London, England) 1975;1(7897):16-9. doi: 10.1016/s0140-6736(75)92376-4
10.1016/s0140-6736(75)92376-4.
59. Badimon JJ, Badimon L, Fuster V. Regression of atherosclerotic lesions by high density lipoprotein plasma fraction in the cholesterol-fed rabbit. J Clin Invest 1990;85(4):1234-41. doi: 10.1172/jci114558.
60. Rubin EM, Krauss RM, Spangler EA, Verstuyft JG, Clift SM. Inhibition of early atherogenesis in transgenic mice by human apolipoprotein AI. Nature 1991;353(6341):265-7. doi: 10.1038/353265a0
10.1038/353265a0.
61. Tangirala RK, Tsukamoto K, Chun SH, Usher D, Pure E, Rader DJ. Regression of atherosclerosis induced by liver-directed gene transfer of apolipoprotein A-I in mice. Circulation 1999;100(17):1816-22. doi: 10.1161/01.cir.100.17.1816.
62. Castelli WP, Doyle JT, Gordon T, Hames CG, Hjortland MC, Hulley SB, Kagan A, Zukel WJ. HDL cholesterol and other lipids in coronary heart disease. The cooperative lipoprotein phenotyping study. Circulation 1977;55(5):767-72. doi: 10.1161/01.cir.55.5.767
10.1161/01.cir.55.5.767.
63. Miller NE, Thelle DS, Forde OH, Mjos OD. The Tromso heart-study. High-density lipoprotein and coronary heart-disease: a prospective case-control study. Lancet (London, England) 1977;1(8019):965-8. doi: 10.1016/s0140-6736(77)92274-7
10.1016/s0140-6736(77)92274-7.
64. Goff DC, Jr., Lloyd-Jones DM, Bennett G, Coady S, D'Agostino RB, Gibbons R, Greenland P, Lackland DT, Levy D, O'Donnell CJ, et al. 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation 2013;129(25 Suppl 2):S49-73. doi: 10.1161/01.cir.0000437741.48606.98
10.1161/01.cir.0000437741.48606.98. Epub 2013 Nov 12.
65. Toth PP, Barter PJ, Rosenson RS, Boden WE, Chapman MJ, Cuchel M, D'Agostino RB, Sr., Davidson MH, Davidson WS, Heinecke JW, et al. High-density lipoproteins: a consensus statement from the National Lipid Association. J Clin Lipidol 2013;7(5):484-525. doi: 10.1016/j.jacl.2013.08.001
10.1016/j.jacl.2013.08.001. Epub 2013 Aug 11.
66. Oldoni F, Sinke RJ, Kuivenhoven JA. Mendelian disorders of high-density lipoprotein metabolism. Circ Res 2014;114(1):124-42. doi: 10.1161/circresaha.113.300634
10.1161/CIRCRESAHA.113.300634.
67. Thompson A, Di Angelantonio E, Sarwar N, Erqou S, Saleheen D, Dullaart RP, Keavney B, Ye Z, Danesh J. Association of cholesteryl ester transfer protein genotypes with CETP mass and activity, lipid levels, and coronary risk. Jama 2008;299(23):2777-88. doi: 10.1001/jama.299.23.2777
10.1001/jama.299.23.2777.
68. Li YY, Wu XY, Xu J, Qian Y, Zhou CW, Wang B. Apo A5 -1131T/C, FgB -455G/A, -148C/T, and CETP TaqIB gene polymorphisms and coronary artery disease in the Chinese population: a meta-analysis of 15,055 subjects. Mol Biol Rep 2012;40(2):1997-2014. doi: 10.1007/s11033-012-2257-9

10.1007/s11033-012-2257-9. Epub 2012 Nov 6.
69. Investigators A-H, Boden WE, Probstfield JL, Anderson T, Chaitman BR, Desvignes-Nickens P, Koprowicz K, McBride R, Teo K, Weintraub W. Niacin in Patients with Low HDL Cholesterol Levels Receiving Intensive Statin Therapy. New England Journal of Medicine 2011;365:2255-67. doi: 10.1056/NEJMoa1107579.
70. Group H-TC, Landray MJ, Haynes R, Hopewell JC, Parish S, Aung T, Tomson J, Wallendszus K, Craig M, Jiang L, et al. Effects of Extended-Release Niacin with Laropiprant in High-Risk Patients. New England Journal of Medicine 2014;371:203-12. doi: 10.1056/NEJMoa1300955.
71. Manninen V, Tenkanen L, Koskinen P, Huttunen JK, Manttari M, Heinonen OP, Frick MH. Joint effects of serum triglyceride and LDL cholesterol and HDL cholesterol concentrations on coronary heart disease risk in the Helsinki Heart Study. Implications for treatment. Circulation 1992;85(1):37-45. doi: 10.1161/01.cir.85.1.37
10.1161/01.cir.85.1.37.
72. Robins SJ, Collins D, Wittes JT, Papademetriou V, Deedwania PC, Schaefer EJ, McNamara JR, Kashyap ML, Hershman JM, Wexler LF, et al. Relation of gemfibrozil treatment and lipid levels with major coronary events: VA-HIT: a randomized controlled trial. JAMA 2001;285:1585-91.
73. Schwartz GG, Olsson AG, Abt M, Ballantyne CM, Barter PJ, Brumm J, Chaitman BR, Holme IM, Kallend D, Leiter LA, et al. Effects of Dalcetrapib in Patients with a Recent Acute Coronary Syndrome. New England Journal of Medicine 2012;367:2089-99. doi: 10.1056/NEJMoa1206797.
74. Lincoff AM, Nicholls SJ, Riesmeyer JS, Barter PJ, Brewer HB, Fox KAA, Gibson CM, Granger C, Menon V, Montalescot G, et al. Evacetrapib and Cardiovascular Outcomes in High-Risk Vascular Disease. New England Journal of Medicine 2017;376:1933-42. doi: 10.1056/NEJMoa1609581.
75. Khera AV, Cuchel M, de la Llera-Moya M, Rodrigues A, Burke MF, Jafri K, French BC, Phillips JA, Mucksavage ML, Wilensky RL, et al. Cholesterol Efflux Capacity, High-Density Lipoprotein Function, and Atherosclerosis. New England Journal of Medicine 2011;364:127-35. doi: 10.1056/NEJMoa1001689.
76. Navab M, Reddy ST, Van Lenten BJ, Fogelman AM. HDL and cardiovascular disease: atherogenic and atheroprotective mechanisms. Nature Reviews Cardiology 2011;8:222-32. doi: 10.1038/nrcardio.2010.222.
77. MacKey RH, Greenland P, Goff DC, Lloyd-Jones D, Sibley CT, Mora S. High-density lipoprotein cholesterol and particle concentrations, carotid atherosclerosis, and coronary events: MESA (Multi-Ethnic Study of Atherosclerosis). Journal of the American College of Cardiology 2012;60:508-16. doi: 10.1016/j.jacc.2012.03.060.
78. Castelli W, Gordon T, Hjortland M, Kagan A, Doyle J, Hames C, Hulley S, Zukel W. ALCOHOL AND BLOOD LIPIDS. The Cooperative Lipoprotein Phenotyping Study. The Lancet 1977;310:153-5. doi: 10.1016/S0140-6736(77)90176-3.
79. Gaziano JM, Buring JE, Breslow JL, Goldhaber SZ, Rosner B, VanDenburgh M, Willett W, Hennekens CH. Moderate alcohol intake, increased levels of high-density lipoprotein and its subfractions, and decreased risk of myocardial infarction. N Engl J Med 1993;329(25):1829-34. doi: 10.1056/NEJM199312163292501.
80. Brien SE, Ronksley PE, Turner BJ, Mukamal KJ, Ghali WA. Effect of alcohol consumption on biological markers associated with risk of coronary heart disease: systematic review and meta-analysis of interventional studies. BMJ : British Medical Journal 2011;342:d636. doi: 10.1136/bmj.d636.

81. Brinton EA. Effects of ethanol intake on lipoproteins. Curr Atheroscler Rep 2012;14(2):108-14. doi: 10.1007/s11883-012-0230-7
10.1007/s11883-012-0230-7.
82. Liisanantti MK, Savolainen MJ. Phosphatidylethanol mediates its effects on the vascular endothelial growth factor via HDL receptor in endothelial cells. Alcohol Clin Exp Res 2008;33(2):283-8. doi: 10.1111/j.1530-0277.2008.00831.x
10.1111/j.1530-0277.2008.00831.x. Epub 2008 Nov 19.
83. Hansel B, Thomas F, Pannier B, Bean K, Kontush A, Chapman MJ, Guize L, Bruckert E. Relationship between alcohol intake, health and social status and cardiovascular risk factors in the Urban Paris-Ile-de-France Cohort: is the cardioprotective action of alcohol a myth? European journal of clinical nutrition 2010;64:561-8. doi: 10.1038/ejcn.2010.61.
84. Foerster M, Marques-Vidal P, Gmel G, Daeppen J-B, Cornuz J, Hayoz D, Pécoud A, Mooser V, Waeber G, Vollenweider P, et al. Alcohol drinking and cardiovascular risk in a population with high mean alcohol consumption. The American journal of cardiology 2009;103:361-8. doi: 10.1016/j.amjcard.2008.09.089.
85. Marques-Vidal P, Bochud M, Paccaud F, Waterworth D, Bergmann S, Preisig M, Waeber Gr, Vollenweider P. No interaction between alcohol consumption and HDL-related genes on HDL cholesterol levels. Atherosclerosis 2010;211:551-7. doi: 10.1016/j.atherosclerosis.2010.04.001.
86. Hiramine Y, Imamura Y, Uto H, Koriyama C, Horiuchi M, Oketani M, Hosoyamada K, Kusano K, Ido A, Tsubouchi H. Alcohol drinking patterns and the risk of fatty liver in Japanese men. Journal of Gastroenterology 2011;46:519-28. doi: 10.1007/s00535-010-0336-z.
87. Anderson KM, Wilson PWF, Garrison RJ, Castelli WP. Longitudinal and secular trends in lipoprotein cholesterol measurements in a general population sample. Atherosclerosis 1987;68:59-66.
88. Langer RD, Criqui MH, Reed DM. Lipoproteins and blood pressure as biological pathways for effect of moderate alcohol consumption on coronary heart disease. Circulation 1992;85:910-5. doi: 10.1161/01.CIR.85.3.910.
89. Koppes LLJ, Twisk JWR, Van Mechelen W, Snel J, Kemper HCG. Cross-sectional and longitudinal relationships between alcohol consumption and lipids, blood pressure and body weight indices. Journal of Studies on Alcohol 2005;66:713-21.
90. Rimm EB, Williams P, Fosher K, Criqui M, Stampfer MJ. Moderate alcohol intake and lower risk of coronary heart disease: Meta-analysis of effects on lipids and haemostatic factors. British Medical Journal 1999;319:1523-8.
91. Criqui MH, Cowan LD, Tyroler HA, Bangdiwala S, Heiss G, Wallace RB, Cohn R. Lipoproteins as mediators for the effects of alcohol consumption and cigarette smoking on cardiovascular mortality: results form the Lipid Research Clinics Follow-up Study. American journal of epidemiology 1987;126:629-37.
92. Suh I. Alcohol Use and Mortality from Coronary Heart Disease: The Role of High-Density Lipoprotein Cholesterol. Annals of Internal Medicine 1992;116:881. doi: 10.7326/0003-4819-116-11-881.
93. Gaziano JM, Buring JE, Breslow JL, Goldhaber SZ, Rosner B, VanDenburgh M, Willett W, Hennekens CH. Moderate Alcohol Intake, Increased Levels of High-Density Lipoprotein and Its Subfractions, and Decreased Risk of Myocardial Infarction. New England Journal of Medicine 1993;329:1829-34. doi: 10.1056/NEJM199312163292501.
94. Djoussé L, Himali JJ, Beiser A, Kelly-Hayes M, Wolf PA. Apolipoprotein e, alcohol consumption, and risk of ischemic stroke: the Framingham Heart Study revisited. Journal

of stroke and cerebrovascular diseases : the official journal of National Stroke Association 2009;18:384-8. doi: 10.1016/j.jstrokecerebrovasdis.2009.01.008.

95. Magnus P, Bakke E, Hoff DA, Høiseth G, Graff-Iversen S, Knudsen GP, Myhre R, Normann PT, Næss Ø, Tambs K, et al. Controlling for high-density lipoprotein cholesterol does not affect the magnitude of the relationship between alcohol and coronary heart disease. Circulation 2011;124:2296-302. doi: 10.1161/CIRCULATIONAHA.111.036491.

Chapter 3

Longitudinal study of alcohol consumption and high-density lipoprotein concentrations: A community-based study

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Longitudinal study of alcohol consumption and high-density lipoprotein concentrations: A community-based study

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Abbreviations used: BMI, body mass index; CRP, C-reactive protein; CVD, cardiovascular disease; HDL, high density lipoprotein; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride.

Abstract

Background: Cross-sectional studies and short-term clinical trials suggest a positive dose-response relationship between alcohol consumption and high-density lipoprotein concentrations. However, prospective data are limited.

Objective: The objective was to determine the association between total alcohol intake and type of alcohol containing beverage, and 6-year (2006-2012) longitudinal change in high density lipoprotein cholesterol (HDL-C) concentrations in a community-based cohort.

Design: This study included 71,379 Chinese adults (mean age 50±11 yr.) who were free of cardiovascular diseases and cancer, and did not use cholesterol-lowering agents during follow-up. Alcohol intake was assessed by a questionnaire in 2006 (baseline) and participants were categorized as never, past, light (women: 0-0.4 servings/d, men: 0-0.9 servings/d), moderate (women: 0.5-1.0 servings/d, men: 1-2 servings/d), and heavy drinkers (women: >1.0 servings/d, men: >2 servings/d). HDL-C concentrations were measured in 2006, 2008, 2010, and 2012. We used generalized estimating equation models to examine the associations between baseline alcohol intake and change in HDL-C concentrations, adjusting for age, sex, smoking, physical activity, obesity, hypertension, diabetes, liver function and C-reactive protein concentrations.

Results: An umbrella-shaped association was observed between total alcohol consumption and changes in HDL-C concentration. Compared with never drinkers, past, light, moderate and heavy drinkers, respectively, experienced a 0.012 (95% CI: 0.008, 0.016), 0.013 (95% CI: 0.010, 0.016), 0.017 (95% CI: 0.009, 0.025), and 0.008 (95% CI: 0.005, 0.011) mmol/L per year slower decrease in HDL-C ($P < 0.0001$ for all) after adjusting for potential confounders. Moderate alcohol consumption was associated with the slowest increase in total cholesterol/HDL-C and triglyceride/HDL-C ratios. We observed a similar association between hard liquor consumption and HDL-C change. In contrast, greater beer consumption was associated with slower HDL-C decreases, in a dose-response manner.

Conclusions: Moderate alcohol consumption was associated with slower HDL-C decreases; however, type of alcoholic beverage had differential effects on change in HDL-C concentration.

Keywords: alcohol, high-density lipoprotein, lipids, cardiovascular disease risk, triglyceride, epidemiology, prospective cohort

Introduction

Previous observational studies consistently reported that individuals with moderate alcohol consumption had a lower risk for cardiovascular disease (CVD), relative to nondrinkers and heavy drinkers (1,2). Consumption of one to two drinks (generally 10-30g alcohol) per day was associated with a 20-25% decrease in risk of coronary heart disease (3–5), however greater alcohol consumptions increased risk of CVD and total mortality (4,5). The beneficial effects of moderate drinking could be attributed to more favorable inflammation and fibrinolytic status, but primarily a result of increased high-density lipoprotein (HDL) concentration (6,7). It was estimated that higher HDL concentration could explain approximately 50% of the coronary heart disease preventive effect of alcohol consumption (8).

Unlike the U-shaped relationship between alcohol consumption and CVD risk, greater alcohol consumption was associated with a higher HDL concentration in a dose-response manner in previous studies(7,9,10). However, most observational studies are cross-sectional (9,11,12), and the long-term impact of alcohol on HDL remains to be elucidated. Experimental studies which have examined the potential effects of a higher intake of alcohol were limited by sample size (n<100) and short follow-up period (<8 weeks) (10). Of note, long-term high alcohol intake (beyond 2 drinks/d) could result in adverse effects such as inflammation, hypertension, hypertriglyceridemia(11), and liver disease (13); the latter two each have well known associations with HDL concentrations and function (13,14). Regardless, in light of recent pharmaceuticals which successfully increased HDL but did not lower risk (19), alcohol remains an important factor in understanding the relationship between HDL and CVD risk.

Alcohol induced increases in triglyceride (TG) could be another factor which mediates the effect of alcohol on CVD risk. The TG/HDL cholesterol (TG/HDL-C) ratio, a well-known predictive biomarker of insulin resistance, is associated with CVD risk (15). Alcohol consumption has been reported to be associated with insulin sensitivity (16,17) and may thus affect the TG/HDL-C ratio. Because there is more cholesterol in the very-low-density lipoprotein in patients with elevated TG concentrations, total/HDL cholesterol (TC/HDL-C) is thought to be preferable to estimate the dyslipidemia status(18). However, to

date, to the best of our knowledge, no study has examined the longitudinal change of these ratios related to alcohol consumption.

In the context, we hypothesized that the association between total alcohol consumption and longitudinal change in HDL-C concentrations was not linear and moderate alcohol consumption was associated with the slowest decline in HDL-C over time. We thus tested this hypothesis in a large community-based cohort including over 70,000 participants with repeated HDL-C measurements. For the secondary analysis, we used changes in other lipid indices including TG/HDL-C ratio and TC/HDL-C ratio as the outcomes. We also assessed the effect of individual alcoholic beverages on HDL-C concentrations and those biomarkers mentioned above.

Subjects and Methods

Study population

We used data from the Kailuan Study, which is an ongoing prospective cohort study conducted in the Kailuan community in Tangshan City in China. In 2006-2007 (i.e., baseline), 101,050 subjects (81,110 men and 20,400 women, age 18-97 yr.) were recruited. All participants completed a standardized questionnaire and underwent physical examinations and laboratory assessments at recruitment. Participants were then followed biennially through 2012 via questionnaires and clinical and laboratory examinations.

In total, 90,299 subjects (89.4%) participated in at least one of the subsequent survey. In the current study, we further excluded participants 1) if they had diagnosed cancer or cardiovascular disease (myocardial infarction or stroke) (n=4,915), 2) if they were reported to use lipids-lowering agents at the baseline or during follow up(n=1,904), 3) if they had no information or incomplete information on alcohol consumption at baseline (n=11, 866), or 4) if they had missing values in surveys on revisit time (n=151), or on HDL-C (n=84). A total of 71, 379 individuals were included in the current analysis (**Figure 1**), and among these participants, 55,371 (77.6%) and 38,089 (53.4%) had 3 and 4 measurements, respectively. Relative to those included in the analyses, participants who were excluded due to missing information on alcohol intake were older (51.3±0.11 vs. 50.3±0.05 yr., $P<0.05$), and had a higher proportion of men (90.7% vs. 76.6%) and smokers (58.3% vs. 31.6%) (Both $P<0.05$), lower LDL-C concentrations (2.28±0.008 vs 2.35±0.003 mmol/L), slightly lower HDL-C concentrations (1.54±0.003 vs. 1.55±0.001 mmol/L, $P<0.05$), and BMI (24.9±0.03 vs. 25.0±0.01 kg/m^2,$P<0.05$) , but similar TG/HDL-C ratios (1.16±0.02 vs. 1.17±0.008, P=0.69), and TC/HDL-C ratios (3.43±0.03 vs. 3.38±0.01, P=0.09). This study was approved by the Ethics Committee of the Kailuan general hospital. All participants provided written informed consent.

Assessment of alcohol consumption

Information on alcohol use was collected using a questionnaire. Participants were asked to report whether they consumed alcoholic beverages in the past 12 months and if so, beverage type (beer, wine, and hard liquor), amount and frequency of intake. Alcohol consumption was calculated in grams per day by multiplying the average frequency (times per day) by usual consuming amount of each beverage and its average ethanol content (5.0 g for 100 g beer, 12.0 g for wine, and 40.0g for hard liquor). A standard drink was approximately 15g of ethanol. According to the number of standard servings, participants were categorized as never, past, light (women: 0-0.4 servings/day, men: 0-0.9 servings/day), moderate (women: 0.5-1.0 servings/day, men: 1-2 servings/day), and heavy drinkers (women: >1.0 servings/day, men: >2 servings/day)(19). For each category of alcoholic beverage, participants who did not drink or did not drink the indicated alcohol type were grouped as "none". Because of the small number of subjects who reported drinking wine (n=311), participants were classified into two groups (yes/no).

Assessment of lipid profiles

Overnight fasting blood samples were drawn from the antecubital vein using vacuum tubes containing EDTA for storage and were repeated collected at the baseline and in the subsequent survey in 2008, 2010 and 2012. Plasma was separated and stored at -80 °C for subsequent analyses. Total cholesterol and triglyceride were both measured using enzymatic colorimetric method (Mind Bioengineering Co. Ltd, Shanghai, China) with an upper limit of detection of 20.68 and 11.30 mmol/L; HDL-C and LDL-C were measured by direct test method (Mind Bioengineering Co. Ltd, Shanghai, China), with respective upper detecting limit of 12.90 and 3.88 mmol/L. Less than 0.1% of measured values were within 5% of either limit. The inter-assay coefficient of variation for each measurement was <10%. All the plasma samples were analyzed using an auto-analyzer (Hitachi 747; Hitachi, Tokyo, Japan) at the central laboratory of the Kailuan General Hospital.

Assessment of potential covariates

Information on age, sex, socio-economic status, lifestyle behaviors, and medical history was collected using questionnaires at baseline. Participants were categorized as never, past, current occasional smoker (<1 cigarette/day), and current daily smoker (≥1 cigarette/day) based on self-report smoking

status. Physical activity was evaluated from responses to questions regarding the frequency of physical activity (20+ minutes per time) during leisure time, with the possible responses including never, sometimes, and 4+ times per week. According to their response, participants were classified as inactive, moderately active and active. Previous studies suggested an inverse association between physical activity levels and risk of developing stroke in the Kailuan study (20).

Body weight and height were measured with participants standing without shoes and outer clothing. Body weight was measured to the nearest 0.1kg using calibrated platform scales, and height was measured to the nearest 0.1 cm using a tape rule. Body mass index was calculated as body weight (kg) divided by the square of height (m^2). Waist circumference was measured in centimeters at the narrowest point between the lowest rib and the iliac crest. Blood pressure was measured twice, at a 5-minutes interval, on the left arm with participants in seated position after at least 5 minutes' rest, using a mercury sphygmomanometer. The average of the two readings was used for analysis. If the two measurements differed by more than 5 mmHg, then a third measurement was taken and the average of the three readings was used for analysis. Hypertension was defined as a systolic blood pressure ⩾140mmHg or a diastolic blood pressure of ⩾90mmHg, or self-report history of hypertension. Prehypertension was defined as a systolic blood pressure ranging between 120 and 140mmHg or a diastolic blood pressure ranging between 80 and 90mmHg.

Fasting blood glucose was measured with the hexokinase/glucose-6-phosphate dehydrogenase method. Plasma high-sensitivity C - reactive protein (hs-CRP) concentrations were measured with high-sensitivity particle-enhanced immunonephelometry assay. Alanine aminotransferase (ALT) was measured with an enzymatic rate method. All the plasma samples were measured using the aforementioned auto-analyzer (Hitachi 747; Hitachi, Tokyo, Japan). Abdominal ultrasonography was performed and interpreted by experienced radiologists blinded to clinical presentation and laboratory findings (HD-15; Philips, Netherlands). Fatty liver was diagnosed and graded as mild, moderate and severe according to ultrasonographic liver features by referring to established criteria (21,22). Diabetes was defined as a

fasting blood glucose of >7.0mmol/L, or a self-report history of diagnosis; pre-diabetes was defined with a fasting blood glucose range of 5.6 mmol/L to 6.9 mmol/L.

Statistical analysis

We used generalized estimating equations (GEE) models to estimate the longitudinal association between alcohol consumption and the change rates in HDL-C and lipid indexes over the 6 years. As noted above, drinking status was categorized into five groups. Never drinkers were taken as the reference group. Three multivariate models were fitted: model 1 adjusting for age and gender; model 2 further adjusting for smoking status, physical activity, body-mass index, presence of hypertension, presence of hyperglycemia, waist circumference, serum concentrations of hs-CRP, and presence of fatty liver; and model 3 further adjusting for TG, TC, and LDL-C where appropriate. Trends in mean differences in HDL-C change rates across total alcohol consumption groups were assessed in GEE models by taking alcohol consumption group number as continuous variable. Associations between alcoholic beverage type and changes in HDL-C were further assessed.

To test the robustness of the main findings, several sensitivity analyses were conducted by excluding smokers, the overweight or obese, hypertensive, diabetics, high CRP (⩾3 mg/l), or those who had fatty liver because all these factors could affect HDL-C concentrations. We also explored potential interactions between alcohol consumption and age, sex, smoking status, physical activity, and waist circumference in relation to changes in HDL-C by adding multiplicative terms in the GEE models.

All the analyses were performed using SAS software, version 9.4 (SAS institute, Cary, NC). P values less than 0.05 was regarded as significant for 2-sided tests.

Results

Of the total participants, 30.7% were current drinkers (16.0% light drinkers, 2.0% moderate drinkers, 12.7% heavy drinkers). Relative to any drinkers, never drinkers had lower prevalence of smoking and lower LDL-C concentration (P<0.001) (**Table 3-1**). As expected, alcohol consumptions were associated with higher baseline HDL-C concentrations. Among those who drank alcohol, there was a dose-response relationship between alcohol consumption and baseline HDL-C concentrations (**Supplemental figure 3-1**).

An umbrella-shaped association was observed between total alcohol consumption and changes in HDL-C concentrations (**Figure 3-2A, and Supplemental table 3-1**), after adjustment for age, sex, and other potential confounders. Consistently, moderate alcohol intake was associated with the slowest increase in TG/HDL-C ratio and TC/HDL-C ratio, across all alcohol drinking groups (**Figure 3-2B and C, and Supplemental table 3-1**). Sensitivity analyses excluding participants with diabetes, hypertension, obesity, fatty liver and elevated CRP, respectively, generated similar results (**Supplemental table 3-2**).

The type of alcohol consumed modified the change in HDL-C concentrations. Mean differences increased in HDL-C (P trend<0.0001), and decreased in TC/HDL-C ratio as beer consumption increased (P trend=0.0007). While for hard liquor consumption, an umbrella-shaped trend was observed for change in both HDL-C concentrations and TC/HDL-C ratios (**Table 3-2**).

We found significant interactions between alcohol intake and age, sex, smoking status, and waist circumference. Compared with never drinkers, HDL-C decreased slowest in light drinkers for women but in moderate drinkers for men. The mean difference in HDL-C change in moderate drinkers was 0.025mmol/L in never/past smokers, and 0.009mmol/L in current smokers, though alcohol intake was still umbrella-shaped related to change in HDL-C (**Supplemental table 3-3**).

Discussion

In this large community-based longitudinal study, we found an umbrella-shaped association between total alcohol consumption and longitudinal change in HDL-C. Decreasing rate in HDL-C concentration was significantly lower with any amount of alcohol intake, compared with never drinkers, but the lowest in moderate alcohol drinkers. Moderate alcohol consumption was also associated with the smallest rise in TC/HDL-C ratio. Exclusion of participants with diabetes, hypertension, fatty liver, or high inflammation status did not materially change the results. The observed nonlinear relation in this study between alcohol intake and HDL-C change during 6 years of follow-up was consistent with the reported U-shaped association between alcohol consumption and CVD risk.

Interestingly, although in a cross-sectional analysis we observed a dose-response relationship between greater alcohol consumptions and higher baseline HDL-C concentrations among alcohol drinkers, which was consistent with previous studies (9–12), the long-term effect of total alcohol consumption on change in HDL-C was nonlinear. One possible explanation is that the heavy drinkers are more likely to develop severe liver diseases, in which case the liver's production of HDL decreased (13). Although adjusting for fatty liver status and alanine aminotransferase did not change the umbrella-shaped relation, alanine aminotransferase may have low sensitivity for alcoholic liver injury (23,24). Furthermore, the impact of alcohol on liver function is generally chronic (23). However, previous observational studies (9,11,12,25,26) on this topic were predominantly cross-sectional, therefore, whether the long-term impact of alcohol consumption on HDL concentration remains a linear pattern could not be inferred. The only two prospective studies on this topic (27,28), reporting a linear relation were based on only two measurements of HDL-C, making it impossible to describe the time-dependent change. Of note, they investigated the association between changes in alcohol consumption, instead of baseline alcohol consumption, and changes in HDL-C, and their analyses should be considered cross-sectional.

Most of the clinical trials regarding alcohol intake and HDL-C concentrations failed to investigate the dose-response relationship and were limited by short term and small sample size ($n<100$) (10,29).

Those trials that examined two or three doses of alcohol intake often targeted only light to moderate doses (30–32). However, two meta-analyses(10,29), using meta-regression approach, reported a dose-response relationship between alcohol consumption and HDL-C concentration. The discrepancy between these meta-analyses of trials and our study could be due to that the clinical trials did not test the long-term effects of high-dose alcohol intake on HDL-C concentration change. The dearth of data limited the ability of the meta-analyses to address the association of alcohol consumption with 6-year change in HDL-C. The dose-response was based on the various doses from different trials, and the estimation of beneficial effects of heavy drinking is questionable. Lastly, heterogeneity in the study design and study population as well as small sample size in each study would lead to greater residual confounding when pooling the data together.

Because an increase in TC concentration is an atherogenic marker, whereas lower HDL-C concentrations is related to risk factors such as metabolic syndrome, the TC/HDL-C ratio is considered as a sensitive index of cardiovascular risk (33). The TG/HDL-C ratio reflects the interaction of overall lipoprotein metabolism and is a useful marker for predicting plasma atherogenicity and insulin resistance(33). The inverse relation of moderate alcohol intake to these lipid indexes confirmed previous findings that light-to-moderate alcohol consumption is associated with reduced risk of coronary heart disease.

We observed that different types of alcoholic beverage had different impacts on change in lipids. Previous studies have generated inconsistent results, with some reporting HDL-C increased more after wine consumption(3,11,34) and some reporting no difference in effect on HDL-C or on CVD risk observed among different types of alcoholic beverage(8–10,35–37). In the current study, because of the small sample size of wine consumers, we dichotomized wine drinking (yes/no) and found that consumption of wine could be protective against HDL-C decrease over time. However, given the low rate of wine intake, the results need to be interpreted with caution. We found diversity of beer and hard liquor consumption in relation to change in HDL-C and lipid ratios. Similar to the relationship observed in total alcohol consumption, moderate consumption of hard liquor was associated with a greater effect on HDL-

C and lipid ratios, whereas higher intake of beer was associated with better lipid profiles. Because we adjusted for alanine aminotransferase and fatty liver diagnosed by ultrasonic wave, it was unlikely that such heterogeneity was totally explained by the confounding caused by the difference in liver function in beer drinkers and liquor drinkers. Another possible explanation is that other components in beer (e.g., polyphenols) could confer more protective effect on reducing CVD risk, and probably HDL-C concentrations (38). Of note, in our study, heavy beer drinkers had lower mean alcohol intake, as compared with heavy hard liquor drinkers (3.2±1.0 vs 4.1±1.8 servings/day).

In this study, we found an average less decrease in HDL of 0.017mmol/l per year (95%CI 0.009-0.025mmol/l) and that would be amounted to 0.102 mmol/l over the 6 years' follow-up. Based on previous data, this would be associated with a risk reduction in CVD of approximately 13.1% (39). The protective effect of alcohol on CVD risk has been recognized as mainly through its role on increase of HDL concentrations (8,40–42). The underlying mechanisms for this protective effect involve alcohol's effect on raising concentrations of HDL and its subfractions (10,42) and on increasing total phospholipid (PL) and polyunsaturated fatty acid content of HDL(7). By affecting the concentration and composition of HDL, and therefore the fluidity, alcohol is thought to promote HDL binding to endothelial cells, reverse cholesterol transport, and up-regulation of vascular endothelial growth factor, protecting against atherosclerosis(43).

Several limitations should be considered. First, carrying some social characteristic, alcohol intake could be underreported, particularly for heavy drinkers, because data on alcohol consumption were collected via self-report. This underreporting might lead to misclassification and underestimate the potential effect of heavy drink. However, social drinking is generally not discouraged in Chinese culture and is generally accepted in men(44), underreporting alcohol consumption would have modest effects on the results if existed. Furthermore, in this study, we did not combine past drinkers with never drinkers, because the time and reason of quitting drinking was not reported. However, compared to never drinkers, past drinkers experienced significantly slower decreasing rate in HDL-C over time. Second, although we controlled most potential confounders, we cannot exclude the possibility of residual confounding. For

example, we did not collect comprehensive dietary intake information. Because moderate drinkers might follow a healthy diet pattern, which is associated with better lipid profiles, we might have overestimated the association between alcohol intake and change in HDL-C concentrations overtime. However, we adjusted for factors such as BMI and waist circumference reflecting dietary intake to some extent. We did not collect information on estrogen therapy until 2010. However, the prevalence of estrogen therapy use was very low in the Kailuan study – in 2010 only 54 women reported to take hormone replacement treatment. Although we cannot exclude the possibility of underreport, the impact of estrogen therapy on the observed alcohol-HDL relationship could be small. At baseline, we did not assess prothrombin time and albumin in this cohort. However, we adjusted for fatty liver status and level of amino transferases, and results did not change materially. Finally, the participants were all from the Kailuan community. Therefore, they could not be viewed as a representative of Chinese population. The results could not be generalized directly to other population with different culture and social-economic backgrounds. However, this geographical confine could greatly reduce residual confounding due to the variance caused by the unmeasured social-economic factors.

In conclusion, our study results suggest an umbrella -shaped relation between total alcohol consumption and longitudinal change in blood HDL-C concentration over a 6-year period, and the decreasing rate was the lowest in individuals consuming light to moderate alcohol. The umbrella-shaped association was mainly driven by hard liquor, not beer, whose association with HDL-C change was linear. These findings support the possible beneficial effect of moderate alcohol on cardiovascular health. More prospective studies are still needed to confirm our findings and to investigate the potential different impact of different alcoholic beverages on lipid metabolism.

Conflict of Interest Statement

No authors reported any disclosure.

Authors' contributions

XG and SW designed the research; JL, XZ, YW and CJ conducted the research; JL, CJ and SH analyzed the data; SH wrote the manuscript; GCS and ALH provided critical study oversight and contributed critical revision of the manuscript for important intellectual content; XG had primary responsibility for the integrity of the data and the accuracy of the data analysis. All authors read and approved the final manuscript.

References

1. Marmot M, Brunner E. Alcohol and cardiovascular disease: the status of the U shaped curve. BMJ. 1991;303:565–8.
2. Maclure M. Demonstration of deductive meta-analysis: ethanol intake and risk of myocardial infarction. Epidemiol Rev [Internet]. 1993;15:328–51. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8174661
3. Di Castelnuovo A, Rotondo S, Iacoviello L, Donati MB, De Gaetano G. Meta-analysis of wine and beer consumption in relation to vascular risk. Circulation. 2002;105:2836–44.
4. Di Castelnuovo A, Costanzo S, Bagnardi V, Donati MB, Iacoviello L, de Gaetano G. Alcohol dosing and total mortality in men and women: an updated meta-analysis of 34 prospective studies. Arch Intern Med [Internet]. 2006;166:2437–45. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17159008
5. Ronksley PE, Brien SE, Turner BJ, Mukamal KJ, Ghali W a. Association of alcohol consumption with selected cardiovascular disease outcomes: a systematic review and meta-analysis. Bmj [Internet]. 2011;342:d671–d671. Available from: http://www.bmj.com/cgi/doi/10.1136/bmj.d671
6. Rimm EB, Moats C. Alcohol and Coronary Heart Disease: Drinking Patterns and Mediators of Effect. Ann Epidemiol [Internet]. Elsevier; 2007;17:S3–7. Available from: http://dx.doi.org/10.1016/j.annepidem.2007.01.002
7. Brinton EA. Effects of ethanol intake on lipoproteins. Curr Atheroscler Rep. 2012;14:108–14.
8. Gaziano JM, Hennekens CH, Godfried SL, Sesso HD, Glynn RJ, Breslow JL, Buring JE. Type of alcoholic beverage and risk of myocardial infarction. Am J Cardiol. 1999;83:52–7.
9. Hansel B, Thomas F, Pannier B, Bean K, Kontush A, Chapman MJ, Guize L, Bruckert E. Relationship between alcohol intake, health and social status and cardiovascular risk factors in the Urban Paris-Ile-de-France Cohort: is the cardioprotective action of alcohol a myth? Eur J Clin

Nutr [Internet]. Nature Publishing Group; 2010;64:561–8. Available from: http://dx.doi.org/10.1038/ejcn.2010.61

10. Brien SE, Ronksley PE, Turner BJ, Mukamal KJ, Ghali WA. Effect of alcohol consumption on biological markers associated with risk of coronary heart disease: systematic review and meta-analysis of interventional studies. BMJ Br Med J [Internet]. 2011;342:d636. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3043110/

11. Foerster M, Marques-Vidal P, Gmel G, Daeppen J-B, Cornuz J, Hayoz D, Pécoud A, Mooser V, Waeber G, Vollenweider P, et al. Alcohol drinking and cardiovascular risk in a population with high mean alcohol consumption. Am J Cardiol [Internet]. Elsevier Inc.; 2009;103:361–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19166690

12. Marques-Vidal P, Bochud M, Paccaud F, Waterworth D, Bergmann S, Preisig M, Waeber G, Vollenweider P. No interaction between alcohol consumption and HDL-related genes on HDL cholesterol levels. Atherosclerosis [Internet]. Elsevier Ireland Ltd; 2010;211:551–7. Available from: http://dx.doi.org/10.1016/j.atherosclerosis.2010.04.001

13. Devenyi P, Robinson GM, Kapur BM, ... High-density lipoprotein cholesterol in male alcoholics with and without severe liver disease. Am J Med [Internet]. 1981;71:589–94. Available from: http://www.sciencedirect.com/science/article/pii/0002934381902114

14. Miller M, Langenberg P, Havas S. Impact of lowering triglycerides on raising HDL-C in hypertriglyceridemic and non-hypertriglyceridemic subjects. Int J Cardiol [Internet]. 2007;119:192–5. Available from: http://www.sciencedirect.com/science/article/pii/S0167527306009867

15. Dobiášová M, Frohlich J. The plasma parameter log (TG/HDL-C) as an atherogenic index: Correlation with lipoprotein particle size and esterification rate inapob-lipoprotein-depleted plasma (FERHDL). Clin Biochem. 2001;34:583–8.

16. Miyake T, Kumagi T, Hirooka M, Furukawa S, Yoshida O, Koizumi M, Yamamoto S, Watanabe T, Yamamoto Y, Tokumoto Y, et al. Low alcohol consumption increases the risk of impaired

glucose tolerance in patients with non-alcoholic fatty liver disease. J Gastroenterol. Springer Japan; 2016;1–11.

17. Marques-Vidal P, Vollenweider P, Waeber G. Alcohol consumption and incidence of type 2 diabetes. Results from the CoLaus study. Nutr Metab Cardiovasc Dis [Internet]. Elsevier B.V; 2015;25:75–84. Available from: http://dx.doi.org/10.1016/j.numecd.2014.08.010

18. Lemieux I, Lamarche B, Couillard C, Pascot A, Cantin B, Bergeron J, Dagenais GR, Després J-P, HR S, SM G, et al. Total Cholesterol/HDL Cholesterol Ratio vs LDL Cholesterol/HDL Cholesterol Ratio as Indices of Ischemic Heart Disease Risk in Men. Arch Intern Med [Internet]. 2001;161:2685. Available from: http://archinte.jamanetwork.com/article.aspx?doi=10.1001/archinte.161.22.2685

19. USDA, USDHHS. Dietary Guidelines for Americans. 2010; Available from: http://health.gov/dietaryguidelines/2010/

20. Zhang Q, Zhou Y, Gao X, Wang C, Zhang S, Wang A, Li N, Bian L, Wu J, Jia Q, et al. Ideal cardiovascular health metrics and the risks of ischemic and intracerebral hemorrhagic stroke. Stroke. 2013;44:2451–6.

21. Saadeh S, Younossi ZM, Remer EM, Gramlich T, Ong JP, Hurley M, Mullen KD, Cooper JN, Sheridan MJ. The utility of radiological imaging in nonalcoholic fatty liver disease. Gastroenterology. 2002;123:745–50.

22. Saverymuttu SH, Joseph AE, Maxwell JD. Ultrasound scanning in the detection of hepatic fibrosis and steatosis. Br Med J (Clin Res Ed) [Internet]. 1986;292:13–5. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1338970&tool=pmcentrez&rendertype=abstract

23. McCullough AJ, O'Connor JFB. Alcoholic liver disease: Proposed recommendations for the American College of Gastroenterology. Am J Gastroenterol. 1998;93:2022–36.

24. O'Shea RS, Dasarathy S, McCullough AJ, Practice Guideline Committee of the American Association for the Study of Liver Diseases, Practice Parameters Committee of the American

College of Gastroenterology. Alcoholic liver disease. Hepatology [Internet]. 2010 [cited 2016 Dec 2];51:307–28. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20034030

25. Wakabayashi I. Increased body mass index modifies associations between alcohol intake and blood cholesterol profile. Eur J Clin Invest. 2012;42:179–85.

26. Park H, Kim K. Association of alcohol consumption with lipid profile in hypertensive men. Alcohol Alcohol. 2012;47:282–7.

27. Anderson KM, Wilson PWF, Garrison RJ, Castelli WP. Longitudinal and secular trends in lipoprotein cholesterol measurements in a general population sample. Atherosclerosis. 1987;68:59–66.

28. Koppes LLJ, Twisk JWR, Van Mechelen W, Snel J, Kemper HCG. Cross-sectional and longitudinal relationships between alcohol consumption and lipids, blood pressure and body weight indices. J Stud Alcohol [Internet]. 2005;66:713–21. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16459932

29. Rimm EB, Williams P, Fosher K, Criqui M, Stampfer MJ. Moderate alcohol intake and lower risk of coronary heart disease: Meta-analysis of effects on lipids and haemostatic factors. Br Med J [Internet]. 1999;319:1523–8. Available from: http://www.scopus.com/inward/record.url?eid=2-s2.0-1942419301&partnerID=40&md5=736d3ea5f30021631caf54d7d4b3d1bd

30. Pikaar NA, Wedel M, van der Beek EJ, van Dokkum W, Kempen HJ, Kluft C, Ockhuizen T, Hermus RJ. Effects of moderate alcohol consumption on platelet aggregation, fibrinolysis, and blood lipids. Metabolism [Internet]. 1987;36:538–43. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2884551

31. Baer DJ, Judd JT, Clevidence BA, Muesing RA, Campbell WS, Brown ED, Taylor PR. Moderate alcohol consumption lowers risk factors for cardiovascular disease in postmenopausal women fed a controlled diet. Am J Clin Nutr. 2002;75:593–9.

32. Hartung GH, Foreyt JP, Reeves RS, Krock LP, Patsch W, Patsch JR, Gotto AM. Effect of alcohol dose on plasma lipoprotein subfractions and lipolytic enzyme activity in active and inactive men.

Metabolism [Internet]. 1990;39:81–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2104642

33. Millan J, Pinto X Fau - Munoz A, Munoz A Fau - Zuniga M, Zuniga M Fau - Rubies-Prat J, Rubies-Prat J Fau - Pallardo LF, Pallardo Lf Fau - Masana L, Masana L Fau - Mangas A, Mangas A Fau - Hernandez-Mijares A, Hernandez-Mijares A Fau - Gonzalez-Santos P, Gonzalez-Santos P Fau - Ascaso JF, et al. Lipoprotein ratios: Physiological significance and clinical usefulness in cardiovascular prevention. 2009;757–65.

34. Marques-Vidal P, Montaye M, Haas B, Bingham A, Evans A, Juhan-Vague I, Ferrieres J, Luc G, Amouyel P, Arveiler D, et al. Relationships between alcoholic beverages and cardiovascular risk factor levels in middle-aged men, the PRIME Study. Prospective Epidemiological Study of Myocardial Infarction Study. Atherosclerosis [Internet]. 2001;157:431–40. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11472744

35. Volcik KA, Ballantyne CM, Fuchs FD, Sharrett AR, Boerwinkle E. Relationship of alcohol consumption and type of alcoholic beverage consumed with plasma lipid levels: differences between Whites and African Americans of the ARIC study. Ann Epidemiol [Internet]. 2008;18:101–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17855114

36. Rimm EB, Klatsky a, Grobbee D, Stampfer MJ. Review of moderate alcohol consumption and reduced risk of coronary heart disease: is the effect due to beer, wine, or spirits. BMJ. 1996;312:731–6.

37. Arranz S, Chiva-Blanch G, Valderas-Martínez P, Medina-Remón A, Lamuela-Raventós RM, Estruch R. Wine, beer, alcohol and polyphenols on cardiovascular disease and cancer. Nutrients [Internet]. 2012 [cited 2016 Apr 15];4:759–81. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22852062

38. Costanzo S, Di Castelnuovo A, Donati MB, Iacoviello L, De Gaetano G. Wine, beer or spirit drinking in relation to fatal and non-fatal cardiovascular events: A meta-analysis [Internet]. European Journal of Epidemiology. 2011 [cited 2016 Apr 15]. p. 833–50. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/22076059

39. Stampfer MJ, Sacks FM, Salvini S, Willett WC, Hennekens CH. A prospective study of cholesterol, apolipoproteins, and the risk of myocardial infarction. N Engl J Med. 1991;325:373–81.

40. Suh I. Alcohol Use and Mortality from Coronary Heart Disease: The Role of High-Density Lipoprotein Cholesterol. Ann Intern Med [Internet]. 1992;116:881. Available from: http://annals.org/article.aspx?articleid=705543

41. Langer RD, Criqui MH, Reed DM. Lipoproteins and blood pressure as biological pathways for effect of moderate alcohol consumption on coronary heart disease. Circulation. 1992;85:910–5.

42. Gaziano JM, Buring JE, Breslow JL, Goldhaber SZ, Rosner B, VanDenburgh M, Willett W, Hennekens CH. Moderate Alcohol Intake, Increased Levels of High-Density Lipoprotein and Its Subfractions, and Decreased Risk of Myocardial Infarction. N Engl J Med [Internet]. 1993;329:1829–34. Available from: http://www.nejm.org/doi/abs/10.1056/NEJM199312163292501

43. Liisanantti MK, Savolainen MJ. Phosphatidylethanol mediates its effects on the vascular endothelial growth factor via HDL receptor in endothelial cells. Alcohol Clin Exp Res [Internet]. 2009 [cited 2016 Apr 15];33:283–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19032580

44. Cochrane J, Chen H, Conigrave KM, Hao W. Alcohol use in China. Alcohol Alcohol [Internet]. The Oxford University Press; 2003 [cited 2016 Apr 9];38:537–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14633640

45. Bild DE, Bluemke DA, Burke GL, Detrano R, Diez Roux A V, Folsom AR, Greenland P, Jacob DR, Kronmal R, Liu K, et al. Multi-Ethnic Study of Atherosclerosis: objectives and design. Am J Epidemiol [Internet]. 2002 [cited 2019 Feb 28];156:871–81. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12397006

46. Cohen R, Gasca NC, McClelland RL, Alcántara C, Jacobs DR, Diez Roux A, Rozanski A, Shea S.

Effect of Physical Activity on the Relation Between Psychosocial Factors and Cardiovascular Events (from the Multi-Ethnic Study of Atherosclerosis). Am J Cardiol [Internet]. 2016 [cited 2018 Nov 6];117:1545–51. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27017324

Table 3-1 Baseline characteristics according to alcohol consumption in 2006[1-3]

		Alcohol consumption					P value
		Never (n = 46902, 65.7%)	Past (n = 2536, 3.6%)	Light (n = 11449, 16.0%)	Moderate (n = 1431, 2.0%)	Heavy (n = 9061, 12.7%)	
Men, %		65.7	97.2	95.5	99.3	99.7	<0.0001
Age(year)		51.3±0.06	53.8±0.23	44.5±0.11	56.4±0.31	50.3±0.12	<0.0001
Body Mass Index (kg/m^2)		25.0±0.02	25.2±0.07	25.2±0.03	24.5±0.09	24.8±0.04	<0.0001
Waist circumstance(cm)		86.7±0.05	86.4±0.19	86.4±0.09	85.2±0.25	86.2±0.10	<0.0001
Physical activity, %	Inactive	4.9	12.9	15.4	10.6	18.6	<0.0001
	Moderately active	83.6	60.7	67.3	53.5	59.8	
	Active	11.5	25.7	17.1	35.8	21.3	
Smoking status, %	Never	85.7	22.0	27.9	21.5	13.2	<0.0001

	Past	1.7	34.3	8.9	10.1	5.7	
	Occasional	1.2	4.5	10.0	3.1	3.3	
	Daily	11.3	39.0	53.2	65.3	77.7	
Diabetes, %	No	73.0	65.9	70.3	70.7	64.9	<0.0001
	Prediabetes	18.5	21.2	23.1	19.9	26.9	
	Yes	8.5	12.9	6.7	9.4	8.3	
Hypertension,%	No	20.8	18.0	27.5	21.1	14.2	<0.0001
	Prehypertension	36.8	33.4	39.2	32.9	37.0	
	Yes	41.9	48.2	33.0	45.7	48.6	
CRP (mg/L)[2]		0.72 (1.00)	0.80 (1.03)	0.79 (1.01)	0.69 (1.04)	0.71 (1.02)	<0.0001
FPG(mmol/l)		5.44±0.01	5.52±0.03	5.42±0.02	5.32±0.04	5.45±0.02	<0.0001
TC (mmol/L)		4.89±0.005	4.90±0.02	4.94±0.01	5.00±0.03	5.17±0.01	<0.0001
LDL-C(mmol/L)		2.28±0.004	2.48±0.02	2.49±0.008	2.44±0.02	2.52±0.01	<0.0001
HDL-C (mmol/L)		1.55±0.002	1.48±0.008	1.50±0.004	1.54±0.01	1.61±0.004	<0.0001
TG (mmol/L)		1.65±0.01	1.56±0.03	1.65±0.01	1.51±0.04	1.80±0.01	<0.0001

[1]Light, man 0.1-0.9 servings/day, woman 0.1-0.4 servings/day; moderate: man 1-2 servings/day, woman 0.5-1.0 servings/day; heavy, man 2+ servings/day, woman 1.0+ servings/day.

[2]Values are mean ± SE adjusted for age and sex (all such values); CRP values are geometric mean (SE). Means were compared by using the general linear model.

[3]CRP, C-reactive protein; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride.

Table 3-2 Mean difference (and 95% CIs) in change rate (mmol/L per year) in HDL-C, TG/HDL-C, and TC/HDL-C during 2006-2012, according to consumption of beer, hard liquor and wine in 2006[1-3]

	Alcohol consumption				P for trend
	None	Light	Moderate	Heavy	
Beer					
N	66806	4483	55	35	
Change in HDL-C	Ref	0.008 (0.005, 0.011)	0.035 (0.012, 0.058)	0.035 (0.010, 0.060)	<0.0001
Change in TG/HDL-C ratio	Ref	0.008 (-0.008, 0.025)	-0.050 (-0.083, -0.017)	-0.059 (-0.131, 0.014)	0.40
Change in TC/HDL-C ratio	Ref	-0.015 (-0.025, -0.005)	-0.068 (-0.124, -0.013)	-0.112 (-0.197, -0.027)	0.0007
Liquor					
N	54132	6794	1444	9009	

Change in HDL-C	Ref	0.014 (0.010, 0.018)	0.016 (0.008, 0.024)	0.007 (0.004, 0.010)	<0.0001
Change in TG/HDL-C ratio	Ref	-0.005 (-0.018, 0.008)	-0.049 (-0.061, -0.038)	0.004 (-0.012, 0.021)	0.70
Change in TC/HDL-C ratio	Ref	-0.021 (-0.031, -0.011)	-0.038 (-0.053, -0.023)	-0.016 (-0.028, -0.005)	<0.0001
Wine[2]					
N	71064		311		
Change in HDL-C	Ref		0.016 (0.005, 0.026)		0.0003
Change in TG/HDL-C ratio	Ref		-0.097 (-0.242, 0.048)		0.21
Change in TC/HDL-C ratio	Ref		-0.024 (-0.070, 0.022)		0.13

[1]All models adjusted for age, sex (men and women), physical activity(inactive, moderately active, and active), smoking status(never, past, occasionally, and daily), diabetics(no, prediabetes, and yes), hypertension(no, prehypertension, and yes), BMI (<24 kg/m^2, 24-28 kg/m^2, 28-30 kg/m^2 and 30+ kg/m^2), waist circumference(<85/90 cm, and ⩾85/90 cm for women/men), C-reactive protein(<1mg/l, 1-3 mg/l, and ⩾3 mg/l), fatty liver(none, mild and severe), plus TG and LDL-C for change in HDL-C, or plus LDL-C for change in TG/HDL-C ratio, or plus TG for change in TC/HDL-C ratio. Generalized estimating equations (GEE) models were used to model the change rates and test the differences in change rates relative to never drinkers.

[2] The data for all of the wine drinkers were presented under moderate group.

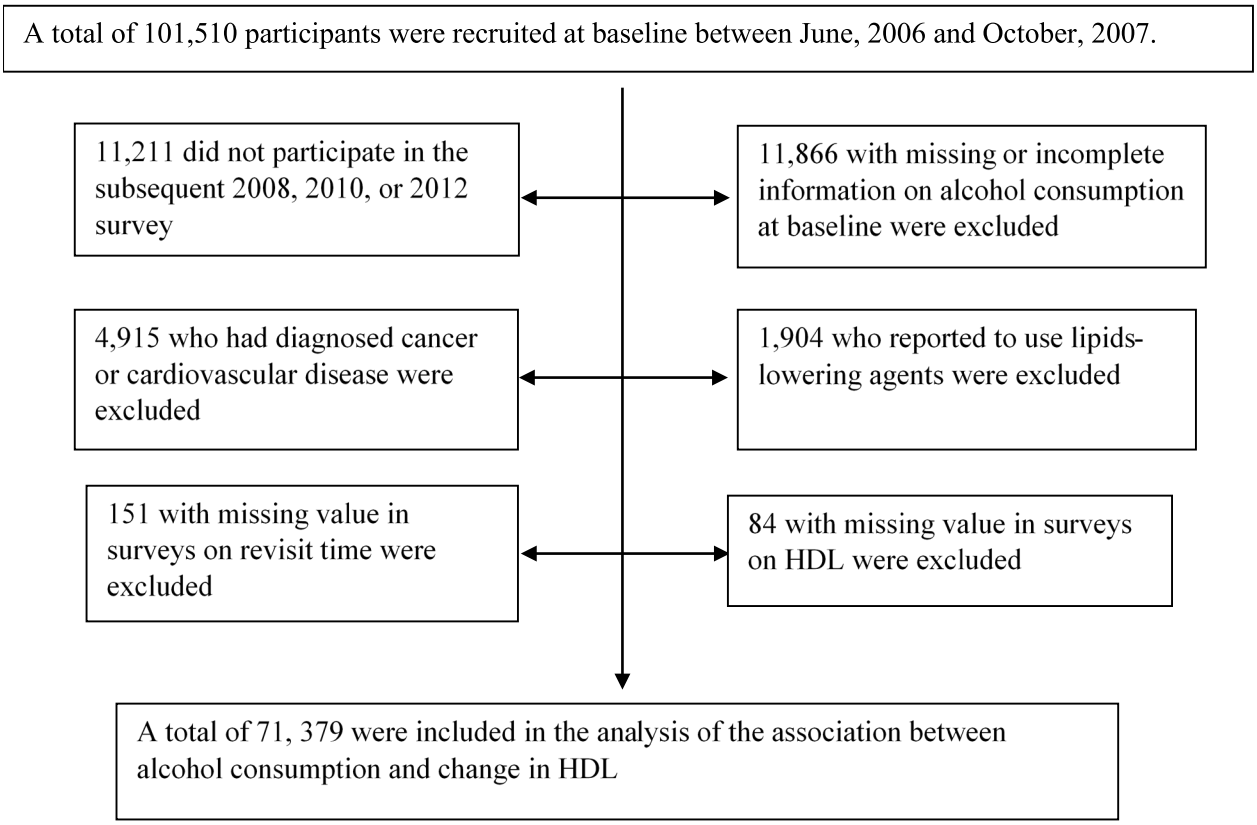
[3] HDL-C, high density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride.

Legends for figures

Figure 3-1: Flow chart of the study

Figure 3-2: Time-dependent change rate (and 95% CIs) in HDL-C concentrations (mmol/L per year) (Panel A), TC/HDL-C (Panel B) and TG/HDL-C ratios (Panel C) according to alcohol consumption. Models were adjusted for age, sex (men and women), physical activity (inactive, moderately active, and active), smoking status (never, past, occasionally, and daily), diabetics (no, prediabetes, and yes), hypertension(no, prehypertension, and yes), BMI (<24 kg/m^2, 24-28 kg/m^2, 28-30 kg/m^2 and 30+ kg/m^2), waist circumference(<85/90 cm, and ⩾85/90 cm for women/men), C-reactive protein(<1mg/l, 1-3 mg/l, and ⩾3 mg/l), fatty liver(none, mild and heavy), low density lipoprotein cholesterol and triglyceride. Participants were classified as never, past, light (women: 0-0.4 servings/d, men: 0-0.9 servings/d), moderate (women: 0.5-1.0 servings/d, men: 1-2 servings/d), and heavy drinkers (women: >1.0 servings/d, men: >2 servings/d) according to alcohol consumption. Generalized estimating equations (GEE) models were used to model the change rates and test the differences in change rates relative to never drinkers. ***P<0.001 relative to never drinkers.

Figure 3-1 Flowchart of the study



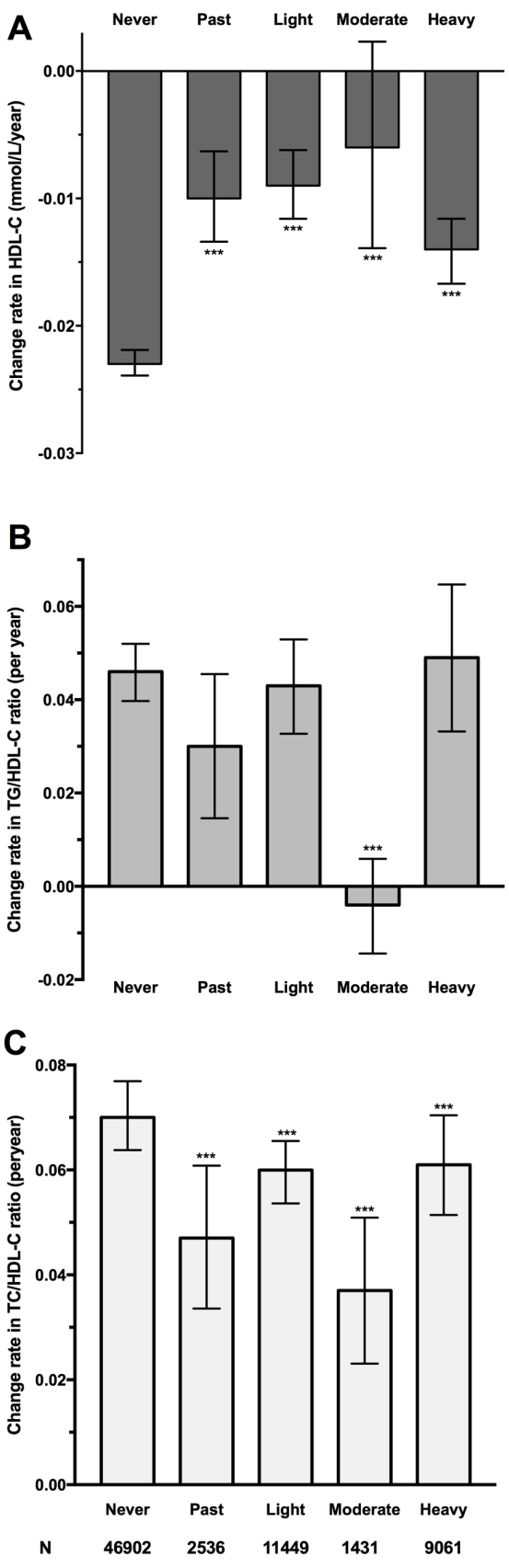


Figure 3-2 Time-dependent change rate in HDL-C concentrations (mmol/L per year; Panel A), TC/HDL-C (per year; Panel B) and TG/HDL-C ratios (per year; Panel C) according to baseline alcohol consumption

Supplemental table 3-1 Mean differences (and 95% CIs) in change rate in HDL-C concentrations (mmol/L per year), TG/HDL-C and TC/HDL-C ratios during 2006-2012, according to alcohol consumption in 2006[1, 2]

		Alcohol consumption					
		Never (n = 46902)	Past (n = 2536)	Light (n = 11449)	Moderate (n = 1431)	Heavy (n = 9061)	P for trend
Change in HDL-C	Model 1	Ref	0.012 (0.008, 0.016)	0.013 (0.010, 0.016)	0.017 (0.009, 0.025)	0.008 (0.005, 0.011)	<0.0001
	Model 2	Ref	0.012 (0.008, 0.016)	0.013 (0.010, 0.016)	0.017 (0.009, 0.025)	0.008 (0.005, 0.011)	<0.0001
	Model 3	Ref	0.013 (0.010, 0.017)	0.014 (0.011, 0.017)	0.017 (0.009, 0.025)	0.009 (0.006, 0.012)	<0.0001
Change in TG/HDL-C ratio	Model 1	Ref	-0.014 (-0.030, 0.002)	-0.0001 (-0.012, 0.012)	-0.051 (-0.063, -0.038)	0.004 (-0.013, 0.021)	0.86
	Model 2	Ref	-0.014 (-0.030, 0.002)	-0.0004 (-0.012, 0.012)	-0.051 (-0.063, -0.038)	0.004 (-0.013, 0.021)	0.85
	Model 3	Ref	-0.016	-0.003	-0.050	0.003	0.92

			(-0.032, 0.001)	(-0.015, 0.009)	(-0.062, -0.038)	(-0.014, 0.020)	
Change in TC/HDL-C ratio	Model 1	Ref	-0.033 (-0.048, -0.018)	-0.019 (-0.028, -0.010)	-0.047 (-0.062, -0.031)	-0.021 (-0.033, -0.009)	0.002
	Model 2	Ref	-0.034 (-0.049, -0.018)	-0.019 (-0.028, -0.010)	-0.047 (-0.062, -0.031)	-0.021 (-0.033, -0.010)	0.002
	Model 3	Ref	-0.034 (-0.049, -0.018)	-0.023 (-0.032, -0.014)	-0.041 (-0.056, -0.025)	-0.021 (-0.032, -0.009)	0.001

[1]Model 1 adjusted for age and sex (men and women); model 2 adjusted for age, sex (men and women), physical activity(inactive, moderately active, and active), smoking status (never, past, occasionally, and daily), diabetics (no, prediabetes, and yes), hypertension (no, prehypertension, and yes), BMI (<24 kg/m2, 24-28 kg/m2, 28-30 kg/m2 and 30+ kg/m2), waist circumference (<85/90 cm, and ⩾85/90 cm for women/men), C-reactive protein(<1mg/l, 1-3 mg/l, and ⩾3 mg/l), fatty liver(none, mild and severe); model 3 adjusted for all the variables in model 2, plus TG and LDL-C for change in HDL-C, or plus LDL-C for change in TG/HDL-C ratio, or plus TG for change in TC/HDL-C ratio. Generalized estimating equations (GEE) models were used to model the change rates and test the differences in change rates relative to never drinkers.

[2]HDL-C, high density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride.

Supplemental table 3-2 Sensitivity analyses for mean differences (and 95% CIs) in change rate in HDL-C concentrations (mmol/L per year), TG/HDL-C and TC/HDL-C ratios during 2006-2012, according to alcohol consumption[1]

		Alcohol consumption					P for trend
		Never	Past	Light	Moderate	Heavy	
Change in HDL-C	Excluding smokers	Ref	0.016 (0.009,0.024)	0.019 (0.014,0.023)	0.027 (-0.004,0.058)	0.014 (0.007,0.021)	<0.0001
	Excluding participants with diabetes	Ref	0.012 (0.008,0.016)	0.012 (0.009,0.015)	0.016 (0.008,0.025)	0.007 (0.005,0.010)	<0.0001
	Excluding participants with hypertension	Ref	0.010 (0.005,0.015)	0.011 (0.007,0.014)	0.014 (0.001,0.028)	0.006 (0.002,0.010)	<0.0001
	Excluding participants with BMI>28 Kg/m^2	Ref	0.013 (0.009,0.017)	0.013 (0.01,0.016)	0.017 (0.008,0.026)	0.009 (0.006,0.012)	<0.0001
	Excluding	Ref	0.010	0.012	0.020	0.007	<0.0001

	participants >60 years		(0.006,0.015)	(0.009,0.015)	(0.008,0.032)	(0.005,0.010)	
	Excluding participants with CRP≥3mg/L	Ref	0.011 (0.007,0.015)	0.012 (0.009,0.015)	0.015 (0.006,0.024)	0.008 (0.005,0.011)	<0.0001
	Excluding participants with higher waist circumference (men>90cm, women >85cm)	Ref	0.013 (0.008,0.018)	0.011 (0.008,0.015)	0.017 (0.004,0.030)	0.008 (0.004,0.012)	<0.0001
	Excluding participants with severe fatty liver	Ref	0.012 (0.008,0.016)	0.013 (0.010,0.015)	0.018 (0.010,0.026)	0.009 (0.006,0.011)	<0.0001
Change in TG/HDL-	Excluding smokers	Ref	-0.034 (-0.051,-0.018)	-0.031 (-0.052,-0.010)	-0.050 (-0.071,-0.030)	0.012 (-0.050,0.073)	0.25

C ratio							
	Excluding participants with diabetes	Ref	-0.022 (-0.036,-0.008)	-0.005 (-0.017,0.007)	-0.050 (-0.061,-0.038)	0.0006 (-0.016,0.017)	0.43
	Excluding participants with hypertension	Ref	-0.021 (-0.039,-0.004)	-0.003 (-0.019,0.012)	-0.043 (-0.059,-0.028)	-0.002 (-0.022,0.017)	0.45
	Excluding participants with BMI>28 Kg/m^2	Ref	-0.016 (-0.034,0.002)	-0.003 (-0.014,0.010)	-0.050 (-0.062,-0.038)	-0.008 (-0.021,0.005)	0.05
	Excluding participants >60 years	Ref	-0.014 (-0.032,0.003)	-0.005 (-0.018,0.009)	-0.050 (-0.066,-0.034)	0.003 (-0.015,0.021)	0.78
	Excluding participants with CRP≥3mg/L	Ref	-0.013 (-0.032,0.007)	-0.001 (-0.013,0.011)	-0.051 (-0.065,-0.038)	0.004 (-0.021,0.012)	0.28

	Excluding participants with higher waist circumference (men>90cm, women >85cm)	Ref	-0.012 (-0.036,0.012)	0.009 (-0.004,0.022)	-0.029 (-0.043,-0.016)	0.003 (-0.013,0.018)	0.67
	Excluding participants with severe fatty liver	Ref	-0.014 (-0.031,0.003)	-0.005 (-0.017,0.007)	-0.050 (-0.062,-0.038)	-0.0004 (-0.016,0.015)	0.37
Change in TC/HDL-C ratio	Excluding smokers	Ref	-0.047 (-0.067,-0.027)	-0.038 (-0.049,-0.026)	-0.029 (-0.056,0.001)	-0.025 (-0.054,0.004)	<0.0001
	Excluding participants with diabetes	Ref	-0.030 (-0.046,-0.014)	-0.024 (-0.033,-0.015)	-0.038 (-0.054,-0.022)	-0.019 (-0.030,-0.008)	<0.0001
	Excluding participants with	Ref	-0.021 (-0.041,-0.002)	-0.018 (-0.027,-0.008)	-0.028 (-0.050,-0.006)	-0.014 (-0.028,0.000)	0.002

hypertension						
Excluding participants with BMI>28 Kg/m^2	Ref	-0.037 (-0.052,-0.022)	-0.023 (-0.033,-0.014)	-0.033 (-0.050,-0.017)	-0.024 (-0.034,-0.013)	<0.0001
Excluding participants >60 years	Ref	-0.031 (-0.049,-0.012)	-0.023 (-0.033,-0.013)	-0.049 (-0.068,-0.030)	-0.019 (-0.032,-0.007)	<0.0001
Excluding participants with CRP≧3mg/L	Ref	-0.027 (-0.043,-0.012)	-0.020 (-0.029,-0.011)	-0.033 (-0.049,-0.017)	-0.021 (-0.033,0.009)	<0.0001
Excluding participants with higher waist circumference (men>90cm,	Ref	-0.034 (-0.052,-0.016)	-0.017 (-0.026,-0.008)	-0.030 (-0.051,-0.010)	-0.015 (-0.028,-0.002)	0.0005

women >85cm)						
Excluding participants with severe fatty liver	Ref	-0.034 (-0.050,-0.019)	-0.023 (-0.032, -0.014)	-0.040 (-0.056,-0.025)	-0.023 (-0.034,-0.012)	<0.0001

[1] All models adjusted for age, sex (men and women), physical activity(inactive, moderately active, and active), smoking status(never, past, occasionally, and daily), diabetics(no, prediabetes, and yes), hypertension(no, prehypertension, and yes), BMI (<24 kg/m^2, 24-28 kg/m^2, 28-30 kg/m^2 and 30+ kg/m^2), waist circumference(<85/90 cm, and ⩾85/90 cm for women/men), C-reactive protein(<1mg/l, 1-3 mg/l, and ⩾3 mg/l), fatty liver(none, mild and severe), plus TG and LDL-C for change in HDL-C, or plus LDL-C for change in TG/HDL-C ratio, or plus TG for change in TC/HDL-C ratio. Generalized estimating equation (GEE) models were used to model the change rates and test the differences in change rates relative to never drinkers.

Supplemental table 3-3 Mean differences (and 95% CIs) in HDL-C decrease rate (mmol/L per year) during 2006-2012 according to alcohol consumption in 2006, stratified by age, sex, smoking status, and waist circumference[1]

		Alcohol consumption					P for trend	P for interaction
		Never	Past	Light	Moderate	Heavy		
Age, year[2]	<60	Ref	0.012 (0.007,0.016)	0.014 (0.011,0.017)	0.019 (0.007,0.032)	0.007 (0.004,0.011)	<0.0001	<0.0001
	⩾60	Ref	0.021 (0.015,0.027)	0.013 (0.008,0.018)	0.018 (0.010,0.026)	0.015 (0.009,0.021)	<0.0001	
Sex[2]	Women	Ref	0.002	0.018	-0.001	0.002	<0.0001	<0.0001

			(-0.017,0.021)	(0.009,0.026)	(-0.032,0.029)	(-0.019,0.024)		
			0.019	0.019	0.023	0.014	<0.0001	
	Men	Ref						
			(0.015,0.023)	(0.016,0.022)	(0.015,0.031)	(0.012,0.017)		
			0.014	0.020	0.025	0.011	<0.0001	
	Never/Past	Ref						0.01
Smoking			(0.009,0.019)	(0.016,0.025)	(0.003,0.047)	(0.006,0.017)		
status[2]			0.008	0.006	0.009	0.003	0.14	
	Current	Ref						
			(0.002,0.014)	(0.002,0.010)	(0.003,0.016)	(-0.001,0.007)		
	<90(man)		0.013	0.014	0.016	0.008	<0.0001	
		Ref						<0.0001
Waist	<85(woman)		(0.008,0.018)	(0.010,0.017)	(0.003,0.029)	(0.004,0.012)		
circumference[2]	90+(man)		0.015	0.014	0.019	0.009	<0.0001	
		Ref						
	85+(woman)		(0.009,0.020)	(0.009,0.019)	(0.011,0.027)	(0.005,0.013)		

[1]All models adjusted for physical activity (inactive, moderately active, and active), diabetics (no, prediabetes, and yes), hypertension (no, prehypertension, and yes), BMI (<24 kg/m2, 24-28 kg/m2, 28-30 kg/m2 and 30+ kg/m2, C-reactive protein (<1mg/l, 1-3 mg/l, and ⩾3 mg/l),

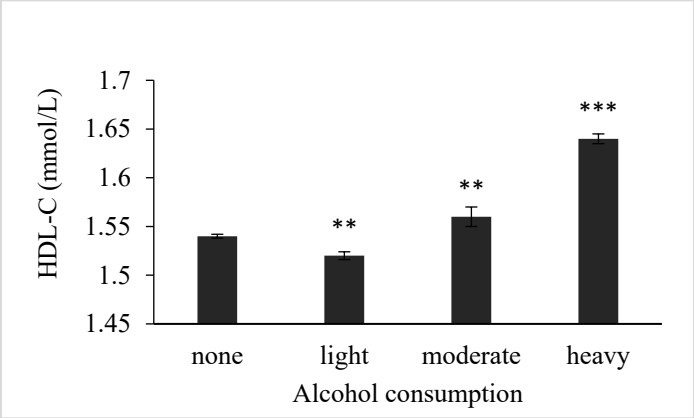
fatty liver (none, mild and severe), LDL-C and TG. Generalized estimating equations (GEE) models were used to model the change rates and test the differences in change rates relative to never drinkers.

[2]Additionally adjusted for sex (men and women), smoking status (never, past, occasionally, and daily), and waist circumference (<85/90 cm, and ≥85/90 cm for women/men) when stratified by age; additionally adjusted for age, smoking status (never, past, occasionally, and daily), and), waist circumference (<85/90 cm, and ≥85/90 cm for women/men) when stratified by sex; ; additionally adjusted for age, sex (men and women) and waist circumference (<85/90 cm, and ≥85/90 cm for women/men) when stratified by smoking status; additionally adjusted for age, sex (men and women) and smoking status (never, past, occasionally, and daily) when stratified by waist circumference.

Legend for supplemental figure

Supplemental figure 3-1 Baseline HDL-C concentration (mmol/L) according to alcohol consumption. Adjusting for age, sex, physical activity, smoking status, diabetics, hypertension, body mass index, waist circumstance, C-reactive protein, fatty liver, low density lipoprotein and triglyceride. Participants were classified as none, light (women: 0-0.4 servings/d, men: 0-0.9 servings/d), moderate (women: 0.5-1.0 servings/d, men: 1-2 servings/d), and heavy drinkers (women: >1.0 servings/d, men: >2 servings/d) according to alcohol consumption. Generalized linear model was used to analyze the data. Generalized linear model was used to compare means and test the trend. **P<0.01, ***P<0.001 relative to none drinkers.

Supplemental figure 3-1 Baseline HDL-C concentration (mmol/L) according to alcohol consumption.



Chapter 4

Alcohol intake and incident myocardial infarction: the role of high density lipoprotein

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Abstract

Objectives: The aim of this study is to test the hypothesis that high-density lipoprotein cholesterol (HDL-C) mediated the association between alcohol intake and myocardial infarction (MI).**Methods:** This study included 81,253 Chinese men and women (mean age: 51±12 yr.) from the Kailuan Study who were free of cardiovascular disease in 2006 (at baseline) and were followed up to Dec. 2016. At baseline, alcohol consumption was assessed via a questionnaire and the concentration of HDL-C was measured. Incident MI at follow up was a first MI event, confirmed by medical record review. Multivariable Cox regression was used to model the association between habitual alcohol intake and risk of MI, adjusting for potential covariates including age, sex, education, monthly income, occupation, smoking status, physical activity, body mass index, waist circumferences, hypertension, diabetes and total cholesterol. Mediated effect through HDL-C was assessed using a causal mediating analysis (SAS macro).

Results: During an average of 9.6 years of follow-up, we documented 1088 incident cases. The adjusted hazard ratio (HR) for MI was 0.74 (95% confidence interval (CI): 0.60, 0.91), 0.80 (95%CI: 0.56, 1.16), 0.56 (95%CI: 0.45, 0.70) for light, moderate, and heavy alcohol drinkers compared with non-drinkers. The ratio changed slightly after further adjustment of HDL-C concentrations. Mediation analysis showed that HDL-C concentrations mediated a small, non-significant proportion (Proportion mediated ~2%) of the effect of alcohol on MI.

Conclusions: Alcohol consumption was associated with a lower risk for MI incidence in a large Chinese cohort. Our results suggest that the benefits on MI associated with moderate alcohol consumption are not mediated by HDL-C.

Introduction

Observational studies have reported that alcohol consumption is inversely associated with risk of myocardial infarction (MI) across many populations (1). Such inverse associations have been attributed to the effect of alcohol on several cardiovascular disease related biomarkers, such as improved inflammation status, and decreased fibrinogen levels, but mainly due to the alcohol-induced increase in high density lipoprotein cholesterol (HDL-C) concentrations (2, 3).

Several observational studies estimating the contributions of HDL-C in mediating the protective effect of alcohol on MI risk have generated controversial results, with some studies suggesting that changes in HDL-C associated with alcohol intake could explain a large proportion of the cardioprotective effect of alcohol (4-10), while other studies reporting that HDL-C is not central in the causal pathway between moderate alcohol intake and cardiovascular diseases (11, 12). The mediating role of HDL-C between alcohol intake and MI remains to be elucidated. In addition, previous studies evaluated mediation from the changes in either beta coefficients (5, 11) or relative risk (RR) (4, 6-10, 12) of alcohol intake before and after adjustment of HDL-C, which generally does not adequately test mediation (13). Compared to this traditional regression approach, the recent causal mediation analysis is more comprehensive and robust because it allows for non-continuous outcomes and exposure-mediator interaction, and also outlines the assumption for causal inference and includes sensitivity analyses for the assumption assessment (14).

In this context, we aimed to investigate the association between alcohol consumption and risk of MI in a large, community-based cohort including over 80,000 Chinese adults, and to test whether HDL-C concentration was mediating in the pathway from alcohol to MI using both the regression the mediation analysis approach.

Methods

Study population

The current study was based on the Kailuan Study (Chinese Clinical Trial Registry number: ChiCTR-TNRC-11001489), which is an on-going prospective cohort study conducted in the Kailuan community in Thangshan City in China. As detailed previously (1, 2), 101,050 participants (81,110 men and 20,400 women, age range: 18-97 yr.) were recruited from the Kailuan community in 2006-2007 (i.e. baseline). At recruitment, all participants underwent a standardized questionnaire interview and physical examination and laboratory assessment. They were followed up biennially on the above measurement through 2016 December. For the data analysis, we excluded participants 1) who had history of diagnosed cardiovascular disease at baseline (n=3,397), 2) who had incomplete information on alcohol intake (n=13,997), 3) who had missing values for HDL cholesterol concentration (n=510), and 4) who had missing values for other covariates (n=2,353), leaving a total of 81, 253 participants included in the current data analysis (**Figure 4-1**). Except for having an older age (age 54±14 y vs 51±12 y), participants included were similar with the excluded in many baseline characteristics including BMI, HDL-C, LDL-C cholesterol, diabetes and hypertension status (**Supplemental table 4-1**).

This study was approved by the Ethics Committee of the Kailuan General Hospital. All participants provided written informed consent.

Assessment of alcohol consumption

Details for assessment of alcohol intake have been described previously (1). Briefly, participants were interviewed by trained field workers using a questionnaire with questions about consumption of alcoholic beverages in the past 12 months, and if so, what type (beer, wine and hard liquor), and the amount and frequency of alcohol intake. Alcohol consumption was

calculated in grams per day by multiplying the average times per day by the usual amount of each beverage and its average ethanol content (5.0 g for 100 g beer, 12.0 g for wine, and 40.0 g for hard liquor). One drink (referred to as "serving" in the report) contains about 14 grams of ethanol (3).

Based on the Dietary Guidelines for Americans 2015-2020, participants were classified into the following groups: non-drinkers, light (women: 0-0.4 servings/d; men: 0-0.9 servings/d), moderate (women: 0.5-1.0 servings/d; men: 1-2 servings/d), and heavy drinkers (women: >1 servings/d; men: >2 servings/d).

Assessment of potential covariates

We collected information on age, sex, education level, per capita monthly income level, occupation, smoking status, and physical activity via a standardized questionnaire in 2006, as detailed elsewhere (4). Height, weight and waist circumference were measured by trained field workers. Body mass index (BMI, in kg/m^2) was calculated as body weight divided by the square of height. Blood pressures were measured according to the 7^{th} Joint National Committee. Hypertension was defined as systolic blood pressure (SBP) of ≥140 mm Hg, or a diastolic blood pressure (DBP) of ≥≥ 90 mm Hg, or usage of anti-hypertensive agents; prehypertension as a SBP of 120 -139 mm Hg or a DBP of 80 -89 mm Hg, and normotension as SBP of < 120 mm Hg and DBP of < 80 mm Hg. Diabetes was defined as fasting blood glucose (FBG) of ≥ 7.0 mmol/l (125mg/dl), or a self-reported history, or the use of hypoglycemic agents; impaired fasting glucose (IFG) was defined as fasting glucose of 5.6 - 6.9 mmol/l (100-125mg/dl).

As detailed previously (1), overnight fasting blood samples were collected, separated, and then stored at -80℃ for subsequent analysis at the Central Laboratory of Kailuan General Hospital. HDL cholesterol and low-density lipoprotein (LDL) cholesterol were measured via a

direct test method (Mind Bioengineering Co. Ltd, Shanghai, China). Total cholesterol and triglyceride were both measured with the use of an enzymatic colorimetric method (Mind Bioengineering Co. Ltd.). Fasting blood glucose was assessed using the hexokinase/glucose-6-phoshate dehydrogenase method.

Incident MI

Incident MI, was defined as the first occurrence of MI events during follow-up, i.e. 2006 to 2016 Dec. To retrieve potential MI cases, the records of surveyed participants were linked to the Municipal Social Insurance Institute and all 11 Kailuan hospitals' discharge registers (2). We used International Classification of Disease, 10th Revision for the classification of potential MI (5). In addition, information regarding medical history of MI was collected by questionnaire biennially after 2006. Potential MI cases identified by the ICD code and/or questionnaire were adjudicated by a panel of three experienced cardiologists. MI was defined based on cardiac enzymes (i.e. levels of creatine kinase and troponin T or I), symptoms, electrocardiographic signs, and necropsy. Fatal MI was confirmed by hospital records or death certificates listing CHD or MI as the main cause of death (6).

Statistical analysis

Categorical variables at baseline were presented as percentage, and continuous data were presented as mean ± standard error. Generalized linear model was used to test difference across groups.

Person-time was calculated as the time difference from the date of baseline examination to either onset of MI, death, or loss of follow-up, or the end of follow-up on Dec 2016. Cox proportional hazard regression models were used to model the association between alcohol consumption and risk of MI. The proportional hazard assumption was satisfied. Hazard ratios

with corresponding 95% CIs were calculated for each alcohol drinking group using non-drinkers as the reference group and for per drink increase in alcohol intake. We fitted four models. In model 1, we adjusted for age and sex; in model 2, we further adjusted education, per capita monthly income, occupation, physical activity, smoking status, and in model 3, we further adjusted family history of MI, blood glucose status, blood pressure status, body mass index, waist circumferences, C-reactive protein, and total cholesterol. In model 4, we further adjusted HDL-C concentration. To compare with previous studies using the traditional regression approach, we used a previously defined equation to calculated the proportion of MI risk reduction explained by HDL-C as $[HR_{\text{model without HDL-C}} - HR_{\text{model with HDL-C}}]/[HR_{\text{model without HDL-C}} - 1]\cdot 100\%$ (7).

To examine the extent to which HDL-C concentration mediates the association between alcohol intake and MI risk, we used a counterfactual approach under the causal framework to decompose the total effect (TE), i.e. the association between alcohol consumption and MI risk, into the natural direct effect (NDE), i.e. the effect of alcohol consumption on MI risk not through HDL-C, and the natural indirect effect (NIE), i.e. the effect of alcohol consumption on MI risk through HDL-C. Before moving forward to mediation analysis, we tested the non-linearity between alcohol consumption and time to MI, HDL-C and time to MI using the SAS %lgtphcurv9 macro (8), which basically tests non-linearity relation by fitting restricted cubic splines. Both the nonlinearity hypothesis were rejected (both P-values >0.1). We estimated the HRs and 95% CI for TE, NDE and NIE by fitting a Cox regression model for the time-to-event outcome and a linear model for the continuous mediator. Alcohol consumption was analyzed first as continuous variable, then as one binary variable to compare participants consuming alcohol to the non-drinkers, and then as 2 binary variables (light versus non-drinkers, and heavy

versus non-drinkers) to explore the importance of different effects of alcohol amount. HDL-C was analyzed as a continuous variable (per 1 mg/dl increase for HDL-C). To reflect the importance of HDL-C at different time points and longitudinal time trend of HDL-C, we further conducted the mediation analysis by including HDL-C measured at different examinations and longitudinal change rate in HDL-C during follow-up as mediators (**Figure 4-2**). We tested the interaction between alcohol intake and baseline HDL-C concentration and we did not find any interaction (P=0.59). However, because interaction is difficult to detect, we included an interaction term in all mediation analysis and reported results both with the interaction and without the interaction for the main analysis.

For sensitivity analysis, we further conducted the above analysis after excluding past drinkers, participants using lipid lowering agents, with diabetes, hypertension, CRP >3mg/L, or BMI > 30 kg/m^2, and coal miners separately. We also explored the potential impact of age, sex and 10-year coronary heart disease risk at baseline on the association between alcohol consumption and risk of MI, and further assessed the association in subgroups stratified by age, sex and Hard Coronary Heart Disease (CHD) risk. We calculated the Hard CHD risk using the risk functions from the Framingham Study official website (9), which is based on The Adult Treatment Panel III (10).

All analyses were performed with the use of SAS software (version 9.4; SAS Institute). $P< 0.05$ was regarded as significant for 2-sided tests.

Results

Among the 81, 253 participants, 66% reported no current alcohol consumption. Compared to non-drinkers, drinkers had higher concentrations of HDL-C, LDL-C, and total cholesterol. They were also more likely to be coal miners, more physically active, current smokers and have IFG (**Table 4-1**).

After an average of 9.6 years of follow-up, we documented 1088 incident cases. The fully adjusted hazard ratios (HRs) for MI was 0.74 (95%CI: 0.60, 0.91), 0.80 (95%CI: 0.56, 1.16), 0.56 (95%CI: 0.45, 0.70) for light, moderate, and heavy alcohol drinkers, compared with non-drinkers (**Table 4-2**). Per drink increase of alcohol intake was associated with an HR of 0.89 (95%CI: 0.84, 0.94) for MI risk. Similar results were observed after excluding past drinkers, participants using lipid lowering agents, with diabetes, hypertension, CRP >3mg/L, or BMI > 30 kg/m^2, and coal miners (**Supplemental table 4-2**). Adding HDL-C in the model barely attenuated the association between alcohol and MI no matter if alcohol intake was treated as categories or as continuous (**Table 4-2**). Calculated mediating proportions from changes in HR were zero for both light and moderate drinkers, and 2.22% for heavy drinkers.

Mediation analysis showed that HDL-C explained a small, non-significant fraction (proportion mediated ~2%) of the association between alcohol intake and MI risk irrespective of alcohol as continuous, one binary or two binary variables (**Table 4-3**), which is similar to the calculated mediation proportion above. Accounting for the alcohol-HDL-C interaction, using follow-up HDL-C, or longitudinal change rate in HDL-C as a mediator, or sensitivity analysis did not change the results materially (**Supplemental table 4-3, 4-4 and 4-5**).

There was no significant interaction between alcohol intake and age, sex and CHD risk score in relation to MI risk (P-interaction>0.05 for all). Similar associations between alcohol intake and MI were observed as the overall population (**Supplemental table 4-6 and 4-7**).

Discussion

In this large prospective cohort study, greater alcohol consumption was related to a lower risk of MI in Chinese adults. Such association was independent of other risk factors for heart disease, such as smoking, physical activity, BMI, diabetes, blood pressure, and total cholesterol. The association between alcohol intake and MI did not change substantially when adding HDL-C into the model. Mediation analysis showed a non-significant slight proportion of HDL-C in mediating the association between alcohol consumption and MI. The results remained unchanged even if excluding past drinkers, participants using lipid-lowering agents, with diabetes, with hypertension, with higher inflammation status of higher BMI, or coal miners.

The current study is, to our knowledge, the first to use a causal inference framework to formally investigate the extent to which HDL contributed to the association between alcohol intake and MI risk. Previous studies (11-19) on this topic have not formally distinguished between HDL-C as a confounder or mediator in the models, and did not provide an estimate for indirect effect through HDL-C and inferential test for the estimate. In the current study, we used the counterfactual frame and formally defined direct and indirect effect of alcohol intake on MI risk and provided both P-values and 95% CI for the indirect effect, i.e. the mediating effect of HDL-C, contributing a robust evidence demonstrating HDL-C is barely mediating the protective effect of alcohol against CHD. Using this approach, we were also able to overcome some limitations of the traditional regression approach such as no interaction allowed. We examined the exposure-mediator, i.e. alcohol-HDL-C interaction in our analysis. We also examined alcohol intake both as continuous variables, binary and categorized variable in the mediation analysis. Our study has several other strengths such as large sample size, detailed alcohol intake information, and comprehensive adjustment of confounders.

Using a similar analytical approach as previous studies (11-19), i.e. comparing the beta coefficients or the relative risk of alcohol before and after adjustment of HDL-C in the regression model, we only found a slight change in the HRs associated with alcohol intake and the risk reduction explained by HDL-C was ~2%. This finding is close to the finding from a Norway cohort study (19) but in contrast to several other prospective cohort studies (11-13, 15). In the Norway cohort study (19) including 71, 225 men and 78, 504 women, Per Magnus et al. found that when comparing men drinking alcohol more than once a week with men never or rarely drinking alcohol, the HR for CHD death changed only slightly (before: 0.52 (0.39,0.69); after: 0.55 (0.41, 0.73)) after adjustment of HDL-C (proportion of risk reduction explained by HDL-C: ~6%). However, in other prospective cohort studies such as Lipid Research Clinics Follow-up Study (LRC) (11), Honolulu Heart Program Study (12), Multiple Risk Factor Intervention Trial (13), and the Nurses' Health Study and Health Professionals follow-up (15), the relative risk/HR reduction explained by HDL-C ranged 30% ~50%.

We have compared the mean HDL-C concentrations of our population with that of populations from previous studies. Except that one study (11) did not report their HDL-C concentrations, the mean HDL-C concentrations of our study population (men: 1.54 mmol/l; women:1.59 mmol/l) are closest to the Norway cohort (men:1.28 mmol/l; women: 1.53 mmol/l) (19) and higher than most other cohorts (men:<1.20 mmol/l; women:<1.43 mmol/l) (12, 13, 15). Emerging Risk Factor Collaboration meta-analysis have reported that there is no statistically significant decrease in CVD risk with HDL-C levels above 1.3 mmol/l (20). Taken together, it seems possible that increasing HDL-C concentration by alcohol would translate into reduced CHD risk only if HDL-C starts at a smaller concentration but not if at a greater concentration.

Therefore, in the current study and the Norway study, adjustment of HDL-C did not result in big change in the HRs related to alcohol.

Careful examination of the analytical approach from previous reports also raised other possible explanations for the discrepant findings among those studies. Because the association between alcohol and CHD risk can be curvilinear in some studies, estimate gained from those studies treating alcohol as a continuous can be inaccurate or invalid. In the Helsinki Heart Study including 1924 middle-aged men (18), Mänttäri M et al. observed a smaller estimate of the proportion of HDL-C in explaining the protective effect for CHD using alcohol as binary variable compared with a continuous variable, i.e. 16% vs 44%. The other several studies reporting larger proportions all treated alcohol as continuous variable although units were different (11-13, 15). Of note, in the NHS and HPFS study, the unit was per drinking day per week (15), and whether the proportion be similarly smaller if alcohol was modeled as a binary remains unknown. Even if the proportion remained greater compared to current study, any negative deviation would enhance our inference that HDL-C is not central in mediating the CHD protective effect of alcohol.

Blood concentration of HDL-C has been associated with a lower risk of cardiovascular diseases (CVDs) in epidemiological studies. However, clinical trials using niacin (21) or CETP inhibitor (22) to raise HDL-C concentration did not successfully reduce risk for CVDs. In the current study, although alcohol intake is related to a lower risk for MI, its effect seems not through HDL-C. This may imply that HDL-C might not be an effective intervention target for CVD prevention, and the effect of alcohol on HDL-C does not necessarily lead to protection against CVD. Therefore, the mechanism of the protective effect of alcohol on CVD remains to be elucidated.

In our study, we found that higher alcohol intake is related to a lower risk for MI, which is consistent with a recent study combined data from 83 cohorts in high-income countries (23). However, the evidence has not always been consistent. In two meta-analyses (24, 25), a U-shaped association was observed between alcohol consumption frequency or quantity and risk of MI or CHD. Noteworthy, one of them included studies from 52 countries worldwide (25) and found that a protective effect of low to moderate alcohol was not observed for studies in South Asian countries while a similar effect of alcohol on MI was observed in studies from Europe/North America/Australia/New Zealand and China or Southeast Asia. As for the risk of CHD, it has been inferred that among regular drinkers (consuming alcohol more than 2 days a week), even high amounts of alcohol intake is related to a lower risk for CHD but not among irregular drinkers (26). In the current study, almost all of the moderate (99.9%) and heavy drinkers (100%) drank every day, which may explain the lower risk.

Our results should be interpreted with caution because of several limitations in this study. First, alcohol consumption was self-reported, and therefore could lead to misclassification. However, because social drinking is widely accepted in Chinese culture (27), especially for men, the impact of misclassification would be modest. Due to the change of questionnaire, we were not able to examine if alcohol intake changed during follow-up. Second, we did not collect information on other components related to HDL properties such as HDL particle numbers, and apolipoproteins, so we cannot examine the role of HDL in the pathway for alcohol and MI comprehensively. Third, our participants have a relatively small proportion of women (20%), and this cohort is living in the Kailuan community, which is geographically confined. Thus, the generalizability of the current study to a different population is limited.

In conclusion, our study suggest that HDL-C is not mediating the association between alcohol intake and risk of MI. Future studies conducted in prospective cohort in different populations using the same approach are needed to confirm our finding. Although alcohol intake is related to a lower risk for MI, in consideration of the global health impact of alcohol, alcohol should not be suggested for MI prevention.

References

1. Wood AM, Kaptoge S, Butterworth AS, Willeit P, Warnakula S, Bolton T, Paige E, Paul DS, Sweeting M, Burgess S, et al. Risk thresholds for alcohol consumption: combined analysis of individual-participant data for 599 912 current drinkers in 83 prospective studies. The Lancet 2018;391:1513-23. doi: 10.1016/S0140-6736(18)30134-X.
2. Brien SE, Ronksley PE, Turner BJ, Mukamal KJ, Ghali WA. Effect of alcohol consumption on biological markers associated with risk of coronary heart disease: systematic review and meta-analysis of interventional studies. BMJ : British Medical Journal 2011;342:d636. doi: 10.1136/bmj.d636.
3. Brinton EA. Effects of ethanol intake on lipoproteins. Curr Atheroscler Rep 2012;14(2):108-14. doi: 10.1007/s11883-012-0230-7
10.1007/s11883-012-0230-7.
4. Criqui MH, Cowan LD, Tyroler HA, Bangdiwala S, Heiss G, Wallace RB, Cohn R. Lipoproteins as mediators for the effects of alcohol consumption and cigarette smoking on cardiovascular mortality: results form the Lipid Research Clinics Follow-up Study. American journal of epidemiology 1987;126:629-37.
5. Langer RD, Criqui MH, Reed DM. Lipoproteins and blood pressure as biological pathways for effect of moderate alcohol consumption on coronary heart disease. Circulation 1992;85:910-5. doi: 10.1161/01.CIR.85.3.910.
6. Suh I. Alcohol Use and Mortality from Coronary Heart Disease: The Role of High-Density Lipoprotein Cholesterol. Annals of Internal Medicine 1992;116:881. doi: 10.7326/0003-4819-116-11-881.
7. Gaziano JM, Buring JE, Breslow JL, Goldhaber SZ, Rosner B, VanDenburgh M, Willett W, Hennekens CH. Moderate Alcohol Intake, Increased Levels of High-Density Lipoprotein and Its Subfractions, and Decreased Risk of Myocardial Infarction. New England Journal of Medicine 1993;329:1829-34. doi: 10.1056/NEJM199312163292501.
8. Mukamal KJ, Jensen MK, Grønbaek M, Stampfer MJ, Manson JE, Pischon T, Rimm EB. Drinking frequency, mediating biomarkers, and risk of myocardial infarction in women and men. Circulation 2005;112:1406-13. doi: 10.1161/CIRCULATIONAHA.105.537704.
9. Rimm EB, Moats C. Alcohol and Coronary Heart Disease: Drinking Patterns and Mediators of Effect. Annals of Epidemiology 2007;17:S3-S7. doi: 10.1016/j.annepidem.2007.01.002.
10. Gaziano JM, Hennekens CH, Godfried SL, Sesso HD, Glynn RJ, Breslow JL, Buring JE. Type of alcoholic beverage and risk of myocardial infarction. The American journal of cardiology 1999;83:52-7. doi: S0002-9149(98)00782-6 [pii].
11. Mänttäri M, Tenkanen L, Alikoski T, Manninen V. Alcohol and coronary heart disease: the roles of HDL-cholesterol and smoking. Journal of internal medicine 1997;241:157-63.
12. Magnus P, Bakke E, Hoff DA, Høiseth G, Graff-Iversen S, Knudsen GP, Myhre R, Normann PT, Næss Ø, Tambs K, et al. Controlling for high-density lipoprotein cholesterol does not affect the magnitude of the relationship between alcohol and coronary heart disease. Circulation 2011;124:2296-302. doi: 10.1161/CIRCULATIONAHA.111.036491.
13. VanderWeele T. Explanation in Causal Inference. 2015:729.
14. McAuley HLRDHJH. Mediation analysis. 2019.
15. Huang S, Li J, Shearer GC, Lichtenstein AH, Zheng X, Wu Y, Jin C, Wu S, Gao X. Longitudinal study of alcohol consumption and HDL concentrations: a community-based study. The American journal of clinical nutrition 2017;105:905-12. doi: 10.3945/ajcn.116.144832.
16. Jin C, Chen S, Vaidya A, Wu Y, Wu Z, Hu FB, Kris-Etherton P, Wu S, Gao X. Longitudinal Change in Fasting Blood Glucose and Myocardial Infarction Risk in a Population Without Diabetes. Diabetes Care 2017;40(11):1565-72. doi: 10.2337/dc17-0610.

17. Odphp. 2015-2020 Dietary Guidelines for Americans. 2015.
18. Huang S, Li J, Wu Y, Ranjbar S, Xing A, Zhao H, Wang Y, Shearer GC, Bao L, Lichtenstein AH, et al. Tea consumption and longitudinal change in high-density lipoprotein cholesterol concentration in Chinese adults. Journal of the American Heart Association 2018;7. doi: 10.1161/JAHA.118.008814.
19. WHO. Internet: https://icd.who.int/browse10/2016/en.
20. Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA, White HD, Executive Group on behalf of the Joint European Society of Cardiology /American College of Cardiology /American Heart Association /World Heart Federation Task Force for the Universal Definition of Myocardial I. Fourth Universal Definition of Myocardial Infarction (2018). J Am Coll Cardiol 2018;72(18):2231-64. doi: 10.1016/j.jacc.2018.08.1038.
21. Djoussé L, Lee IM, Buring JE, Gaziano JM. Alcohol consumption and risk of cardiovascular disease and death in women: Potential mediating mechanisms. Circulation 2009;120:237-44. doi: 10.1161/CIRCULATIONAHA.108.832360.
22. Ruifeng Li, Ellen Hertzmark, Mary Louie, Linlin Chen, Spiegelman D. The SAS LGTPHCURV9 Macro 2011.
23. Framingham Heart Study
24. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). Jama 2001;285(19):2486-97. doi: 10.1001/jama.285.19.2486.
25. Di Angelantonio E, Sarwar N, Perry P, Kaptoge S, Ray KK, Thompson A, Wood AM, Lewington S, Sattar N, Packard CJ, et al. Major lipids, apolipoproteins, and risk of vascular disease. JAMA - Journal of the American Medical Association 2009;302(18):1993-2000. doi: 10.1001/jama.2009.1619.
26. Group H-TC, Landray MJ, Haynes R, Hopewell JC, Parish S, Aung T, Tomson J, Wallendszus K, Craig M, Jiang L, et al. Effects of Extended-Release Niacin with Laropiprant in High-Risk Patients. New England Journal of Medicine 2014;371:203-12. doi: 10.1056/NEJMoa1300955.
27. Lincoff AM, Nicholls SJ, Riesmeyer JS, Barter PJ, Brewer HB, Fox KAA, Gibson CM, Granger C, Menon V, Montalescot G, et al. Evacetrapib and Cardiovascular Outcomes in High-Risk Vascular Disease. New England Journal of Medicine 2017;376:1933-42. doi: 10.1056/NEJMoa1609581.
28. Corrao G, Bagnardi V, Zambon A, La Vecchia C. A meta-analysis of alcohol consumption and the risk of 15 diseases. Preventive Medicine 2004;38:613-9. doi: 10.1016/j.ypmed.2003.11.027.
29. Leong DP, Smyth A, Teo KK, McKee M, Rangarajan S, Pais P, Liu L, Anand SS, Yusuf S. Patterns of alcohol consumption and myocardial infarction risk: Observations from 52 countries in the INTERHEART case-control study. Circulation 2014;130:390-8. doi: 10.1161/CIRCULATIONAHA.113.007627.
30. Bagnardi V, Zatonski W, Scotti L, La Vecchia C, Corrao G. Does drinking pattern modify the effect of alcohol on the risk of coronary heart disease? Evidence from a meta-analysis. Journal of Epidemiology & Community Health 2008;62:615-9. doi: 10.1136/jech.2007.065607.
31. Cochrane J, Chen H, Conigrave KM, Hao W. Alcohol use in China. Alcohol Alcohol 2003;38(6):537-42. doi: 10.1093/alcalc/agg111.

Table 4-1 Baseline characteristic by alcohol consumption in 2006 in the Kailuan Cohort (N=81, 253)[1-3]

		Non-drinkers	light	moderate	high
N		n=56910	n=12500	n=1657	n=10186
Sex, %					
Men		69.6	95.8	99.4	99.7
Baseline age, y		52±12 [2]	46±13	58±11	52±10
BMI, kg/m^2		25.0±3.6	25.3±3.5	24.7±3.3	25.0±3.2
Waist circumferences, cm		86.7±10.3	86.7±9.3	87.5±9.6	87.6±9.1
C-reactive protein[3], mg/L		0.79 (4.67)	0.73 (4.22)	0.80 (4.31)	0.73 (4.35)
HDL-C, mmol/l		1.56±0.40	1.47±0.37	1.56±0.40	1.61±0.41
LDL-C, mmol/l		2.28±0.88	2.51±0.80	2.43±0.90	2.54±0.90
Total cholesterol, mmol/l		4.92±1.13	4.89±1.14	5.02±1.22	5.17±1.22
Education, %	Illiterate	9.2	8.4	23.2	15.9
	Middle	76.3	50.4	58	67.6
	College	14.5	41.2	18.8	16.5
Income (RMB/month), %	<600	21.8	39.5	38.7	44.5
	600-1000	68	35.6	42.4	38.5

	>1000	10.2	24.9	18.8	17
Occupation, %	White collar	6.1	14.9	7.5	5.1
	Coal miner	20	45.3	48.5	55.4
	Other blue collar	73.9	39.9	44	39.5
	Inactive	5.2	15.2	10.6	18.2
Physical activity, %	Moderately active	82.5	67.4	53.9	59.7
	Active (4+times/wk)	12.3	17.5	35.6	22.1
Cigrette smoking, %	never	81.9	27.6	21.8	13.1
	past	3.7	9.4	9.8	5.9
	current	14.4	63	68.4	81
Diabetes status, %	Normoglycemia	71.3	69.2	70.5	64
	IFG	18.9	23.3	20.2	27.2
	DM	9.8	7.5	9.4	8.8
Hypertention status, %	normotensive	19.1	26.5	19.7	13.5
	prehypertension	36.1	38.1	33.3	36.2
	HTN	44.9	35.3	47.1	50.4

[1] Participants were classified into the following categories of alcohol consumption: non-drinkers, light (women: 0-0.4 drinks/d; men: 0-0.9 drinks/d), moderate (women: 0.5-1.0 drinks/d; men:1-2 drinks/d), and heavy (women: >1.0 drinks/d; men: > 2drinks/d).

[2] Mean ± SD (all such values unless otherwise indicated). Means were compared with the use of a general linear model, adjusted for age and sex.

[3] Values are geometric means (SD). Means were compared with the use of a general linear model, adjusted for age and sex.

Table 4-2 Adjusted hazard ratios (HRs) and 95% confidence intervals (95% CIs) for risk of myocardial infarction by alcohol consumption (N=81, 253)[1]

	Alcohol consumption				P for trend
	None	light	moderate	heavy	
Case/N	818/56910	125/12500	32/1657	113/10186	
Person-years	543069	119403	15975	98213	
Incident rate/1,000 person-years	1.51	1.05	2.00	1.15	
Model 1	Ref (1)	0.82 (0.68, 0.99)	0.90 (0.63, 1.28)	0.72 (0.59, 0.88)	0.0007
Model 2	Ref (1)	0.73 (0.60, 0.90)	0.77 (0.54, 1.11)	0.60 (0.48, 0.74)	<0.0001
Model 3	Ref (1)	0.74 (0.60, 0.91)	0.80 (0.56, 1.16)	0.56 (0.45, 0.70)	<0.0001
Model 4	Ref (1)	0.74 (0.60, 0.91)	0.81 (0.56, 1.16)	0.57 (0.45, 0.71)	<0.0001

[1] Model 1 adjusted age, sex (men or women); model 2 adjusted variables from model, plus education(illiterate, middle, or college), income(<600, 600-1000, or >1000RMB/mo), occupation (white collar, coal miner, or other blue collar), physical activity (inactive, moderate, or active), smoking status (never, past, or current); model 3 adjusted variables from model 2, plus , blood glucose status (normoglycemia, prediabetes, or diabetes), blood pressure status (normotensive, prehypertension, or hypertension), body mass index (in kg/m2; <24, 24-27.9, 28-29.9, or ≥30), waist circumferences (<85 or ≥85 cm for women, and <90 or ≥90 cm for men), C-reactive protein (<1, 1-2.9, or ≥3mg/L), total cholesterol (<5.2, 5.2-6.2, and ≥6.2 mmol/l); model 4 further adjusted HDL cholesterol.

Table 4-3 Natural direct and indirect effect of alcohol intake on MI and the proportion mediated through HDL-C in the Kailuan cohort (N=81253)[1-2]

	HR^{NDE} (95% CI)	P-value	HR^{NIE} (95% CI)	P-value	HR^{TE} (95% CI)	P-value	Proportion mediated
Alcohol in 2006 -> HDL-C in 2006 -> MI							
Exposure: continuous alcohol intake							
One drink per day	0.89 (0.84, 0.94)	<0.0001	0.998 (0.995, 1.00)	0.12	0.89 (0.84, 0.93)	<0.0001	1.6%
Exposure: binary alcohol intake							
Yes versus no	0.66 (0.56, 0.78)	<0.0001	0.995 (0.989, 1.00)	0.11	0.66 (0.56, 0.78)	<0.0001	1.0%
Exposure: alcohol intake level							
Light-to-moderate versus no	0.75 (0.61, 0.90)	0.0028	0.999 (0.997, 1.00)	0.26	0.74 (0.61, 0.90)	0.0027	0.3%
Heavy versus no	0.58 (0.46, 0.72)	<0.0001	0.987 (0.97,1.00)	0.1	0.57 (0.45, 0.71)	<0.0001	1.8%

[1] NDE: natural direct effect; NIE: natural indirect effect; TE: total effect

[2] All models adjusted age, sex (men or women), education(illiterate, middle, or college), income(<600, 600-1000, or >1000RMB/mo), occupation (white collar, coal miner, or other blue collar), physical activity (inactive, moderate, or active), smoking status (never, past, or current), blood glucose status (normoglycemia, prediabetes, or diabetes), blood pressure status (normotensive, prehypertension, or hypertension), body mass index(in kg/m2; <24, 24-

27.9, 28-29.9, or ≥30), waist circumstance(<85 or ≥85 cm for women, and <90 or ≥90 cm for men), C-reactive protein (<1, 1-2.9, or ≥3mg/L), and total cholesterol (<5.2, 5.2-6.2, and ≥6.2 mmol/l).

Legends for figures

Figure 4-1: Flowchart of the study

Figure 4-2: Mediation analysis of the effects of HDL-C concentration on the association between alcohol consumption and MI

Figure 4-1. Flowchart of the study

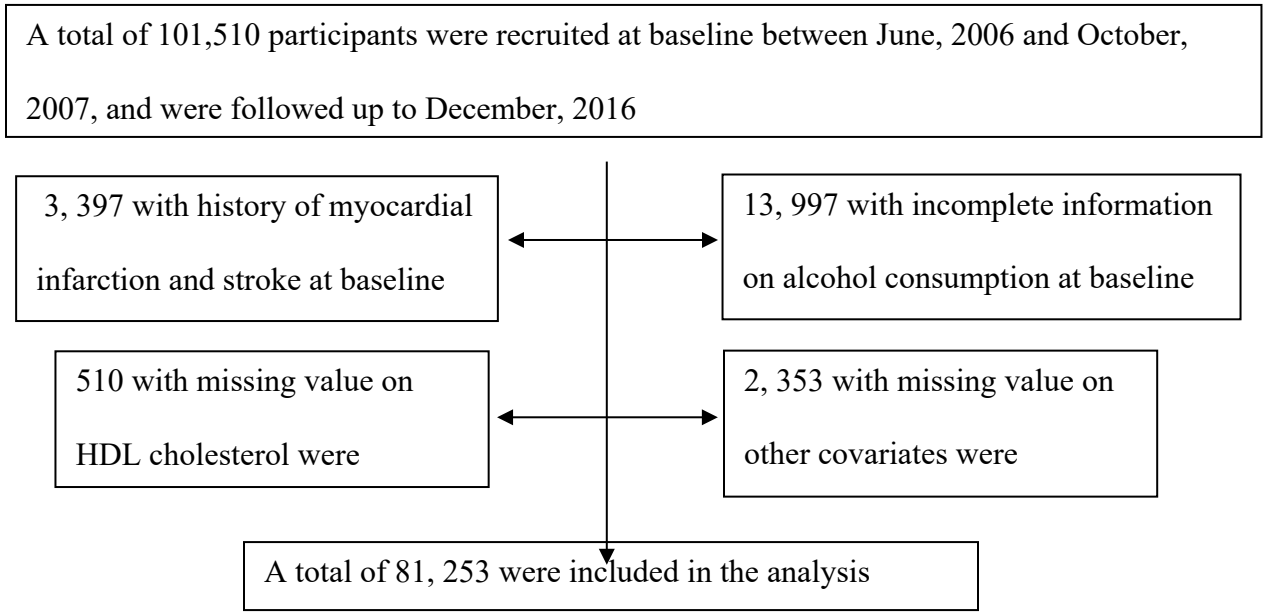
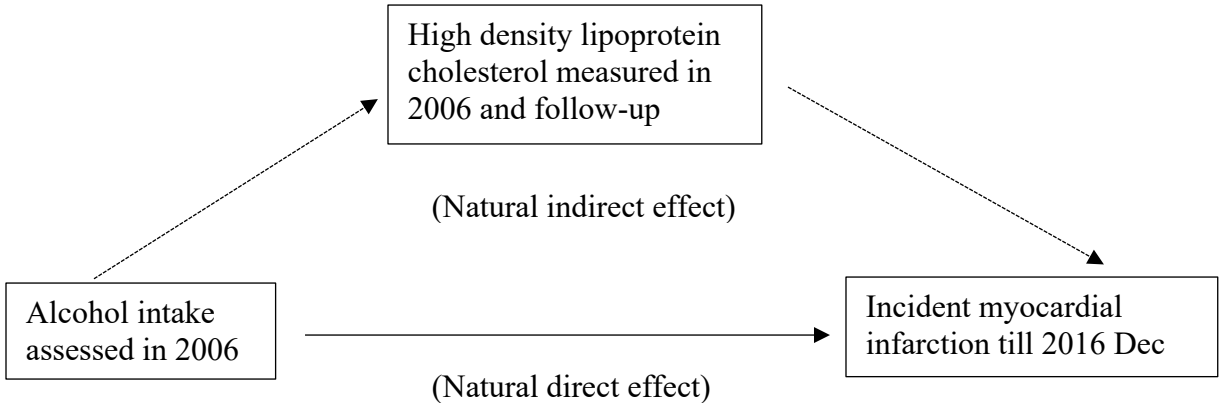


Figure 4-2 Mediation analysis of the effects of HDL-C concentration on the association between alcohol consumption and MI



Supplemental table 4-1 Baseline characteristics between included and excluded in 2006 in the Kailuan Cohort [1]

		Included	excluded
N		n=81253	n=20257
Sex, %			
Men		78	87.6
baseline age, y		51±12 [2]	54±14
BMI, kg/m^2		25.1±3.5	25.0±3.5
Waist circumferences, cm		86.8±10.0	88.2±10.1
C-reactive protein[3], mg/L		0.77 (4.57)	1.02 (4.90)
HDL-C, mmol/l		1.55±0.40	1.53±0.42
LDL-C, mmol/l		2.35±0.88	2.32±1.04
Total cholesterol, mmol/l		4.95±1.16	4.95±1.11
triglyceride		1.36 (1.84)	1.35 (1.86)
Education, %	Illiterate	10.2	14.6
	Middle	70.8	59.5
	College	18.9	25.9
Income (RMB/month), %	<600	27.7	35.3
	600-1000	58.8	46
	>1000	13.5	18.7
Occupation, %	White collar	7.3	9.7
	Coal miner	63.7	51.7
	Other blue collar	28.9	38.6
Physical activity, %	Inactive	8.5	10.1

	Moderately active	76.7	69.7
	Active (4+ times/wk)	14.8	20.2
Cigarette smoking, %	never	63.7	40.5
	past	5	10.5
	current	31.3	49.1
Diabetes status, %	Normoglycemia	70.1	68.3
	IFG	20.6	19.8
	DM	9.3	11.9
Hypertension status, %	Normotensive	19.5	19.5
	Prehypertension	36.3	29.9
	HTN	44.1	50.6

[1] Participants were classified into the following categories of alcohol consumption: non-drinkers, light (women: 0-0.4 drinks/d; men: 0-0.9 drinks/d), moderate (women: 0.5-1.0 drinks/d; men:1-2 drinks/d), and heavy (women: >1.0 drinks/d; men: > 2drinks/d).

[2] Mean ± SD (all such values unless otherwise indicated). Means were compared with the use of a general linear model, adjusted for age and sex.

[3] Values are geometric means (SD). Means were compared with the use of a general linear model, adjusted for age and sex.

Supplemental table 4-2 Sensitivity analysis for adjusted hazard ratios (HRs) and 95% confidence intervals (95% CIs) for risk of myocardial infarction by alcohol consumption[1]

	Alcohol consumption			
	None	light	moderate	heavy
excluding past drinkers	Ref (1)	0.72 (0.58, 0.89)	0.78 (0.54, 1.12)	0.55 (0.43, 0.68)
excluding lipid lowering	Ref (1)	0.73 (0.59, 0.90)	0.80 (0.56, 1.15)	0.57 (0.45, 0.70)
excluding diabetes	Ref (1)	0.69 (0.55, 0.88)	0.87 (0.60, 1.28)	0.60 (0.47, 0.76)
excluding hypertension	Ref (1)	0.73 (0.51, 1.03)	0.90 (0.51, 1.61)	0.58 (0.40, 0.85)
excluding CRP> 3mg/L	Ref (1)	0.67 (0.53, 0.86)	0.75 (0.48, 1.16)	0.55 (0.42, 0.71)
excluding BMI> 30kg/m^2	Ref (1)	0.72 (0.57, 0.91)	0.89 (0.61, 1.29)	0.60 (0.47, 0.76)
excluding coal miners	Ref (1)	0.76 (0.58, 1.00)	0.63 (0.37, 1.09)	0.54 (0.39, 0.73)

[1] All models adjusted age, sex, education, income, occupation, physical activity, smoking status, BMI, waist circumferences, blood glucose status, blood pressure status, CRP, total cholesterol

Supplemental table 4-3 Natural direct and indirect effect of alcohol intake on MI and the proportion mediated through HDL-C in the Kailuan cohort accounted for alcohol-HDL-C interaction (N=81, 253)[1-2]

	HR^{NDE} (95% CI)	P-value	HR^{NIE} (95% CI)	P-value	HR^{TE} (95% CI)	P-value	Proportion mediated
Alcohol in 2006 -> HDL-C in 2006 -> MI							
Exposure: continuous alcohol intake							
One drink per day	0.89 (0.85, 0.94)	<.0001	0.998 (0.995, 1.00)	0.094	0.89 (0.84, 0.94)	<.0001	1.6%
Exposure: binary alcohol intake							
Yes versus no	0.66 (0.56, 0.78)	<.0001	0.992 (0.98, 1.00)	0.20	0.66 (0.56, 0.78)	<.0001	1.5%
Exposure: alcohol intake level							
Light-to-moderate versus no	0.75 (0.62, 0.90)	0.0029	0.9997 (0.995, 1.00)	0.90	0.75 (0.61, 0.90)	0.0029	0.1%
Heavy versus no	0.58 (0.46, 0.74)	<.0001	0.97 (0.93,1.01)	0.15	0.57 (0.45, 0.71)	<.0001	4.0%

[1] NDE: natural direct effect; NIE: natural indirect effect; TE: total effect

[2] All models adjusted age, sex (men or women), education(illiterate, middle, or college), income(<600, 600-1000, or >1000RMB/mo), occupation (white collar, coal miner, or other blue collar), physical activity (inactive, moderate, or active), smoking status (never, past, or current), blood glucose status (normoglycemia, prediabetes, or diabetes), blood pressure status (normotensive, prehypertension, or hypertension), body mass index(in kg/m2; <24, 24-27.9, 28-29.9, or ≥30), waist circumstance(<85 or ≥85 cm for women, and <90 or ≥90 cm for men), C-reactive protein (<1, 1-2.9, or ≥3mg/L), and total cholesterol (<5.2, 5.2-6.2, and ≥6.2 mmol/l).

Supplemental table 4-4 Natural direct and indirect effects of alcohol intake on MI and the proportion mediated through HDL-C during follow-up and change in HDL-C in the Kailuan Cohort[1-3]

	Case/N	HR^{NDE} (95% CI)	P-value	HR^{NIE} (95% CI)	P-value	HR^{TE} (95% CI)	P-value	Proportion mediated
HDL-C at Survey 2 (2008)	713/60394	0.57 (0.45, 0.74)	<.0001	1.00 (0.995, 1.01)	0.41	0.58 (0.45, 0.74)	<.0001	-0.4%
HDL-C at Survey 3 (2010)	437/55354	0.52 (0.39, 0.68)	<.0001	0.99 (0.96, 1.03)	0.60	0.51 (0.39, 0.67)	<.0001	1.1%
HDL-C at Survey 4 (2012)	275/55354	0.54 (0.37, 0.77)	0.0007	0.99 (0.95, 1.02)	0.45	0.53 (0.37, 0.76)	0.0005	1.7%
Change rate in HDL-C	891/72530	0.64 (0.43,0.95)	0.03	0.995 (0.98, 1.01)	0.53	0.64 (0.43, 0.95)	0.03	0.9%

[1] NDE, natural direct effect; NIE, natural indirect effect; HR, hazard ratio; TE, total effect

[2] Hazard ratios for the effect of alcohol intake yes vs no (reference group).

A causal inference framework for mediation analysis was used to estimate HR and 95% CI for total, natural direct and indirect effects. The natural direct and indirect effects were estimated by fitting a Cox regression model for the time-to-event outcome, and a linear regression model for the continuous mediator. From these combined models HR of natural direct and indirect effects were derived. Total effects are equal to the product of the natural direct and indirect effects.

The proportion mediated was calculated as $[HR^{NDE} (HR^{NIE} – 1)] / [HR^{NDE} \times HR^{NIE} – 1] \times 100\%$, and approximates the extent to which the effect of the alcohol on time-to-MI is mediated through HDL-C relative to the overall effect of the alcohol.

Alcohol-HDL-C interaction was accounted for.

[3] Adjusted for age, sex (men or women), education(illiterate, middle, or college), income(<600, 600-1000, or >1000RMB/mo), occupation (white collar, coal miner, or other blue collar), physical activity (inactive, moderate, or active), smoking status (never, past, or current), family history of MI (yes or no), blood glucose status (normoglycemia, prediabetes, or diabetes), blood pressure status (normotensive, prehypertension, or hypertension), body mass index(in kg/m2; <24, 24-27.9, 28-29.9, or ≥30), waist circumstance(<85 or ≥85 cm for women, and <90 or ≥90 cm for men), C-reactive protein (<1, 1-2.9, or ≥3mg/L), total cholesterol (<5.2, 5.2-6.2, and ≥6.2 mmol/l), and baseline HDL-C.

Supplemental table 4-5 Sensitivity analysis on natural direct and indirect effect of alcohol intake on MI and the proportion mediated through HDL-C in the Kailuan cohort

	HR^{NDE} (95%CI)	P-value	HR^{NIE} (95% CI)	P-value	HR^{TE} (95%CI)	P-value	Proportion mediated
Alcohol in 2006 -> HDL-C in 2006 -> MI							
Exposure: binary alcohol intake							
Excluding past drinkers	0.65 (0.54, 0.77)	<.0001	0.99 (0.98, 1.004)	0.2	0.64 (0.54, 0.76)	<.0001	1.8%
Excluding lipid lowering	0.66 (0.56, 0.78)	<.0001	0.99 (0.98, 1.005)	0.21	0.66 (0.56, 0.78)	<.0001	1.9%
Excluding diabetes	0.67 (0.56, 0.81)	<.0001	0.99 (0.98, 1.005)	0.23	0.67 (0.56, 0.80)	<.0001	2.0%
Excluding hypertension	0.69 (0.52, 0.91)	0.008	0.99 (0.97, 1.01)	0.37	0.68 (0.51, 0.90)	0.0062	2.2%
Excluding CRP > 3 mg/L	0.63 (0.52, 0.76)	<.0001	0.99 (0.97, 1.004)	0.16	0.62 (0.51, 0.75)	<.0001	1.7%
Excluding BMI > 30 kg/m^2	0.68 (0.57, 0.82)	<.0001	0.99 (0.98, 1.004)	0.18	0.68 (0.57, 0.81)	<.0001	2.1%
Excluding coal miners	0.65 (0.52, 0.81)	0.0002	0.99 (0.98, 1.003)	0.19	0.64 (0.51, 0.80)	0.0001	1.8%

[1] NDE, natural direct effect; NIE, natural indirect effect; HR, hazard ratio; TE, total effect

[2] Hazard ratios for the effect of alcohol intake yes vs no (reference group).

A causal inference framework for mediation analysis was used to estimate HR and 95% CI for total, natural direct and indirect effects. The natural direct and indirect effects were estimated by fitting a Cox regression model for the time-to-event outcome, and a linear regression

model for the continuous mediator. From these combined models HR of natural direct and indirect effects were derived. Total effects are equal to the product of the natural direct and indirect effects.

The proportion mediated was calculated as $[HR^{NDE} (HR^{NIE} – 1)] / [HR^{NDE} \times HR^{NIE} – 1] \times 100\%$, and approximates the extent to which the effect of the alcohol on time-to-MI is mediated through HDL-C relative to the overall effect of the alcohol.

Alcohol-HDLC interaction was accounted for.

[3] Adjusted for age, sex (men or women), education(illiterate, middle, or college), income(<600, 600-1000, or >1000RMB/mo), occupation (white collar, coal miner, or other blue collar), physical activity (inactive, moderate, or active), smoking status (never, past, or current), family history of MI (yes or no), blood glucose status (normoglycemia, prediabetes, or diabetes), blood pressure status (normotensive, prehypertension, or hypertension), body mass index(in kg/m2; <24, 24-27.9, 28-29.9, or ≥30), waist circumstance(<85 or ≥85 cm for women, and <90 or ≥90 cm for men), C-reactive protein (<1, 1-2.9, or ≥3mg/L), total cholesterol (<5.2, 5.2-6.2, and ≥6.2 mmol/l), and baseline HDL-C.

Supplemental table 4-6 Adjusted hazard ratios (HRs) and 95% confidence intervals (95% CIs) for risk of myocardial infarction by alcohol consumption in subgroups (N=81, 253)[1]

		Alcohol consumption			
		None	light	moderate	heavy
Men	Case/N	708/39587	124/11977	32/1647	113/10156
	Person-years	378427	114520	15882	97928
	Incident rate/1,000 person-years	1.87	1.08	2.01	1.15
	model	Ref (1)	0.74 (0.60, 0.92)	0.82 (0.57, 1.19)	0.57 (0.46, 0.71)
Women	Case/N	110/17323	1/523	0/10	0/30
	Person-years	164642	4883	93	285
	Incident rate/1,000 person-years	0.67	0.20		
	model	Ref (1)	0.33 (0.04, 2.39)	-	-
age≥60	Case/N	386/13488	30/1490	16/684	43/1771
	Person-years	130176	14620	6695	17295
	Incident rate/1,000 person-years	2.97	2.05	2.39	2.49
	model	Ref (1)	0.68 (0.46, 1.01)	0.76 (0.45, 1.27)	0.74 (0.53, 1.05)

age<60	Case/N	432/43422	95/11010	16/973	70/8415
	Person-years	412893	104783	9280	80918
	Incident rate/1,000 person-years	1.05	0.91	1.72	0.87
	model	Ref (1)	0.77 (0.60, 0.99)	0.85 (0.51, 1.42)	0.46 (0.35, 0.62)
Framingham score					
CHD risk>5%	Case/N	511/19375	85/5407	24/1174	84/6544
	Person-years	185353	51844	11326	63120
	Incident rate/1,000 person-years	2.75	1.64	2.12	1.33
	model	Ref (1)	0.72 (0.56, 0.92)	0.77 (0.51, 1.18)	0.54 (0.42, 0.70)
Framingham score					
CHD risk<5%	Case/N	307/37535	40/7093	8/483	29/3642
	Person-years	357716	67559	4649	35093
	Incident rate/1,000 person-years	0.86	0.59	1.72	0.83
	model	Ref (1)	0.74 (0.51, 1.07)	0.93 (0.45, 1.92)	0.56 (0.36, 0.86)

[1] All models adjusted age, sex, education, income, occupation, physical activity, smoking status, BMI, waist circumferences, blood glucose status, blood pressure status, CRP, and total cholesterol when appropriate for the stratified variable.

Supplemental table 4-7 Mediation analysis on alcohol, HDL-C, and MI in subgroups by sex, age and Framingham score (N=81, 253)[1]

		Natural direct effect		Natural indirect effect		Total effect		
		HR	95% CI	HR	95%CI	HR	95%CI	Proportion mediated
Men	model 1	0.842	(0.78, 0.91)	0.996	(0.990, 1.00)	0.84	(0.78, 0.90)	2.1%
	model 2	0.897	(0.85, 0.94)	0.997	(0.994, 1.00)	0.90	(0.85, 0.94)	2.5%
Women	model 1	0.24	(0.01, 6.93)	1.002	(0.994, 1.01)	0.24	(0.01, 6.94)	- 0.1%
	model 2	-	-	-	-	-	-	-
age>=60	model 1	0.91	(0.81, 1.02)	0.997	(0.990, 1.00)	0.91	(0.81, 1.02)	2.9%
	model 2	0.973	(0.89, 1.06)	0.997	(0.992, 1.00)	0.97	(0.89, 1.05)	9.8%
age<60	model 1	0.802	(0.73, 0.88)	0.997	(0.991, 1.00)	0.80	(0.73, 0.88)	1.2%
	model 2	0.852	(0.80, 0.91)	0.998	(0.994, 1.00)	0.85	(0.80, 0.91)	1.1%
Framingham score								
CHD risk>5%	model 1	0.83	(0.76, 0.90)	0.996	(0.989, 1.00)	0.83	(0.76, 0.90)	1.9%
	model 2	0.884	(0.83, 0.94)	0.998	(0.994, 1.00)	0.88	(0.83, 0.94)	1.5%

Framingham score								
CHD risk<5%	model 1	0.86	(0.75, 0.99)	1	(0.995, 1.01)	0.86	(0.75, 0.99)	0.0%
	model 2	0.91	(0.83, 1.00)	0.998	(0.993, 1.00)	0.91	(0.82, 1.00)	2.0%

[1] All Models adjusted age, sex (men or women), education(illiterate, middle, or college), income(<600, 600-1000, or >1000RMB/mo), occupation (white collar, coal miner, or other blue collar), physical activity (inactive, moderate, or active), smoking status (never, past, or current), blood glucose status (normoglycemia, prediabetes, or diabetes), blood pressure status (normotensive, prehypertension, or hypertension), body mass index(in kg/m^2; <24, 24-27.9, 28-29.9, or ≥30), waist circumstance(<85 or ≥85 cm for women, and <90 or ≥90 cm for men), C-reactive protein (<1, 1-2.9, or ≥3mg/L), and total cholesterol when appropriate for the stratified variables. In model 1 alcohol consumption was fitted as a continuous variable using the number of drinking group; model 2 adjusted same variables in model 1, in model 2, alcohol consumption was fitted as continuous variable using number of drinks per day.

Chapter 5

High density lipoprotein particle concentration, not HDL cholesterol mediates the effect of alcohol intake on myocardial infarction: the Multi-Ethnic Study of Atherosclerosis

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Abstract

Objectives: The aim of this study was to investigate whether and to what extent the association between alcohol intake and incident myocardial infarction (MI) is mediated through high density lipoprotein cholesterol (HDL-C), and HDL particles (HDL-P).

Methods: A total of 6,683 participants from the Multi-Ethnic Study of Atherosclerosis were included in the analysis. Alcohol consumption information was assessed via a questionnaire at baseline (exam 1). HDL-C concentrations and HDL-P were both measured at exam 1. Cox regression was used to model the association of habitual alcohol intake and risk for MI before and after adjusting HDL-C and HDL-P, in addition to adjustment of all potential covariates. Mediated effects through HDL-C, and HDL-P were estimated using the causal mediation analysis.

Results: After a median of 8 years follow-up, 171 incident MI cases were documented. Higher alcohol intake was associated with a lower risk for incident MI (P for trend =0.039). The relation between alcohol intake and MI was slightly attenuated by adjustment of HDL-C and moderately by HDL-P. Mediation analysis showed no mediating effect of HDL-C (HR^{NIE}: 0.98; 95%CI: 0.94, 1.02; P-value=0.26), whereas HDL-P slightly mediated the effect of habitual alcohol intake on MI (HR^{NIE}: 0.95, 95%CI: 0.90, 0.99; P-value=0.027). The proportion of the total effect of alcohol on MI mediated by HDL-P was 15.8%.

Conclusions: This study indicates that the lower risk of MI related to alcohol intake appears to partially work through increasing HDL-P; however, the mechanism for much of alcohol's effect to reduce MI risk remains unexplained. This suggests that HDL-P can be a target for MI prevention, however the mediating effect of HDL-P is very moderate.

Introduction

Previous observational studies have suggested that high density lipoprotein cholesterol (HDL-C) is the mediator for the effect of alcohol consumption on coronary heart diseases (CHD) (1-6). In contrast to a 50% increase in the beta coefficient for alcohol intake in some studies (1-3, 7), other study (8) found that including HDL-C in the Cox regression model did not change the hazard ratio of alcohol intake. In addition, clinical trials successfully increasing HDL-C concentration using niacin (9, 10), or cholesteryl ester transfer protein (CETP) inhibitor (11-14) failed to reduce cardiovascular disease risk. Therefore, the role of HDL-C as intervention target for CHD prevention remains inconclusive.

HDL is a complex particle composed of a heterogeneous group of small discoid and spherical particles, including different kinds of apolipoproteins such as apoA-I, and apo C, and lipids such as triglycerides and cholesterol esters. The commonly used HDL-C, which has long been used as a biomarker for assessing CHD risk (15-18), represents only the cholesterol content of HDL. Its increase can result from multiple pathways including an increase in HDL particle number, and increase in the cholesterol content per HDL particle or both(19). Therefore, HDL-C may not be the most appropriate metric to assess the relationship between HDL metabolism and atherosclerosis. Alternative index such as HDL particle number (HDL-P), or apoA-I has been proposed to better represent the main functional property of HDL (20, 21). However, few studies have examined the role of the alternative index in mediating the association between alcohol intake and risk for CHD.

The present study was conducted to examine if HDL-C, HDL-P or apoA-I mediates the association between alcohol intake and risk of MI in a multi-ethnic population, when controlling all potential confounders.

Methods

Study Population

The Multi-Ethnic Study of Atherosclerosis (MESA) is a prospective cohort study designed to investigate the prevalence, risk factors, and progression of subclinical cardiovascular disease in a multi-ethnic cohort in the United States. The study design and methods have been described previously (22). Briefly, from 2000 to 2012, 6814 non-pregnant participants aged 45-84y were recruited from 6 US communities: Forsyth County, NC; New York, NY; Baltimore, MD; St Paul, MN; Chicago, IL; and Los Angeles, CA. Each site recruited an approximately equal number of men and women, according to the pre-specified age, gender, and race/ethnicity proportions. Baseline exclusion criteria included self-reported cardiovascular diseases (heart attack, angina, stroke, transient ischemic attack, heart failure, current atrial fibrillation, history of CVD related procedures including coronary artery bypass grafting (CABG), angioplasty, valve replacement, pacemaker or defibrillator implantation and any other surgery on the heart or arteries), cancer, weight over 300 pounds, and cognitive inability. The MESA study was approved by the institutional review board from all participating study sites. All participants gave informed consent. For the current analysis, we further excluded participants with incomplete information on alcohol consumption (n=59), on HDL-cholesterol concentrations or particle concentrations (n=38), on follow-up time and incident myocardial infarction (n=34), leaving 6,683 participants included (**Figure 5-1**).

A subcohort of the participants (n=4,679) were enrolled in one of the ancillary study to have their apolipoproteins measured (23). After excluding individuals with missing information on the variables, 4592 individuals were included for the analysis on apoA-I.

Alcohol consumption Assessment

Details of alcohol consumption assessment in the MESA cohort have been described previously (24). Briefly, participants were asked if they have ever consumed alcoholic beverages, and if yes, they were asked if they currently drink alcoholic beverages. Participants were categorized into never, former, and current drinkers based on these two questions. Both former and current drinkers were further asked about the usual number of drinks consumed per week. One drink means one beer (a 12 oz. glass, can, or bottle) or one glass of wine (4 oz.) or one shot of spirits (1.5 oz.). Current drinkers were also asked about the number of drinks consumed during the past 24 h, and the largest number of drinks consumed in 1d in the past month. Current drinkers were categorized into: light (women: 0-0.5 drinks/day; men: 0-1 drinks/day), moderate (women: 0.5-1 drinks/day; men: 1-2 drinks/day), and heavy (women: > 1 drinks/day; men: > 2 drinks/day) drinkers.

Lipid, lipoprotein and other Laboratory Assay

Lipids were measured in EDTA plasma, using the CDC/NHLBI standards. HDL-C and total cholesterol was measured using the cholesterol oxidase method (Roche Diagnostics, Indianapolis). The laboratory coefficient of variation were 2.9% and 1.6%, separately. LDL-cholesterol is calculated using the formula of Friedewald et al (25). Plasma lipoprotein particle concentrations were measured using the NMR LipoProfile-II spectroscopy (LipoScience, Inc.; Raleigh, NC). Calculated values for mean LDL and HDL particle size were also provided. Serum glucose was measured using the glucose oxidase method (Johnson & Johnson Clinical Diagnostics, Inc., Rochester, NY 14650). ApoA-Ilevels were measured in the laboratory of Dr. Alan Remaley (Bethesda, MD) using Diazyme reagents and quantified using a Siemens Dimension analyzer (23). CRP was measured via a particle enhanced immunonepholometric assay using the BNII nephelometer (N High Sensitivity CRP; Dade Behring Inc., Deerfield, IL) at the Laboratory for Clinical Biochemistry Research. Fasting blood was collected and processed.

Lipids, lipoprotein and glucose were measured at the Collaborative Studies Clinical Laboratory at Fairview-University Medical Center (Minneapolis, MN).

Myocardial Infarction ascertainment

For the current study, the participants were followed for incident cardiovascular events from baseline until the participant's follow-up 10 telephone interview. At intervals of 9-12 months, a telephone interviewer contacted each participant to inquire about all interim hospital admissions and cardiovascular outpatient diagnoses and procedures. Trained personnel abstracted any hospital records suggesting possible cardiovascular events. Two physicians classified the endpoint and if disagreement occurred, the full review committee made the final classification. In the current study, we used incident myocardial infarction as the endpoint, including definite or probable myocardial infarction. Definite or probable MI required either abnormal cardiac biomarkers regardless of pain or echocardiographic findings; evolving Q waves regardless of pain or biomarker findings; or a combination of chest pain, and ST-T evolution or new left bundle branch block, and abnormal levels of biomarkers.

Measurement of other variables

Information on demographics, other lifestyle behaviors such as smoking, physical activity, and medical conditions were collected via questionnaire at the baseline examination. Cigarette smoking status was defined as never, former (smoke 100 cigarettes in lifetime) and current (smoked cigarettes in last 30 days). Physical activity was measured using a detailed, semi-quantitative questionnaire adopted from the Cross-Cultural Activity Participation Study (45). Total intentional exercise was computed as the sum of walking for exercise, sports/dancing, and conditioning metabolic equivalent (MET) –minutes. Height and weight were measured directly. Body mass index was calculated (in kg/m^2). Resting blood pressure was measured 3 times in the seated position, and the average of the last 2 readings was used. Hypertension was defined as a diastolic blood pressure $\geqslant$ 140 mmHg, or diastolic blood pressure $\geqslant$ 90 mmHg, or self-reported

hypertension and anti-hypertensive medication use. Diabetes was defined as a fasting glucose concentration > 125 mg/dl or use of diabetes medication.

Statistical Analysis

Means and SDs were calculated for selected continuous variables; proportions were calculated for categorical variables. General linear model was used to test differences of continuous variables across groups. Person-years were calculated as the time difference from the date of baseline examination to either the event time (onset of MI), censor time (death or loss of follow-up), or the end of follow-up (Follow-Up 10). Cox models were used to estimate the association between alcohol intake and risk of MI. The proportional hazard assumption was tested by including product terms of the predictors and a function of survival time, and it was satisfied (P=0.52). Hazard ratios with corresponding 95% CIs were calculated for each alcohol drinking group compared to non-drinkers. We fitted 6 models. In model 1, we adjusted age, sex and race; in model 2, we adjusted variables from model 1, plus smoking status, education level (≥/<high school), income level (≥/< $ 25,000) , total intentional exercise (scaled down by dividing its standard deviation because of the wide range and then stratified into tertiles (46); in model 3, we adjusted variables from model 2, plus blood glucose status, hypertension status, body mass index, waist circumferences, C-reactive protein, and total cholesterol; in model 4, we further adjusted HDL-C; in model 5, we adjusted variables from model 3 plus HDL-P numbers; and in model 6, we adjusted variables from model 3 plus both HDL-C and HDL-P. For apoA-I, we replicated the analysis with the aforementioned 3 models, and a model 4 with further adjustment of apoA-Iin the subcohort individuals (n=4592).

We used a counterfactual approach under the causal framework to decompose the total effect of alcohol intake (assessed in 2000-2002) on prospective development of MI (followed to the 10th Follow-Up) into natural direct and indirect effects through HDL (**Figure 5-2**). In order to have a causal interpretation, the mediation analyses need to meet several assumptions, i.e. there

are no uncontrolled factors related to both the exposure and the outcomes. Models in the mediation analysis have been adjusted for baseline covariates as mentioned above in the Cox model. Those confounding factors were selected based on their association with both the exposure, mediator, and the outcome.

Analyses were performed by using the approach developed by Vanderweele for time-to-event outcomes (26). Alcohol consumption was analyzed first as one binary variable to compare participants consuming alcohol to the non-drinkers, and then as 2 binary variables (light versus non-drinkers, and heavy versus non-drinkers) to explore the importance of different effects of alcohol amount. HDL-C, HDL-P and apoA-Iwere analyzed as a continuous variable (per 1 mg/dl increase for HDL-C, per 1 umol/l for HDL-P and per 1 mg/dl increase for apoA-I). The importance of HDL-C at different time points was examined by including HDL-C as a mediator at baseline or at different examinations during follow-up. The natural direct and indirect effects were estimated by fitting a Cox regression model for the time-to-event outcome (conditional on the exposure, mediator, an exposure-mediator interaction depending on its significance, and a set of confounders), and a linear model for the continuous mediator (conditional on the exposure and a set of confounders). From these combined models, we derived the HRs and 95% CIs for the natural direct effect, i.e. the effect of alcohol consumption on MI risk not through HDL-C, HDL-P or apoA-I; the natural indirect effect, i.e. the effect of alcohol consumption on MI risk through HDL-C or HDL-P or apoA-I; and total effect, i.e., the association between alcohol consumption and MI risk. Although we did not observe any interaction between alcohol intake and HDL-C (P=0.80), HDL-P (P=0.83), or apoA-I (P=0.91) in the preliminary regression analyses, interactions are known to be difficult to detect because of limited power. Therefore, we report both the results with or without interaction term in the mediation analysis. The proportion of the exposure-outcome relation that is mediated through HDL-C, HDL-P or apoA-I was computed on a risk difference scale assuming the outcomes of interest are considered rare outcomes(27, 28):

$$Proportion\ mediated = [HR^{NDE}\ (HR^{NIE} - 1)] \div [HR^{NDE} \times HR^{NIE} - 1] \times 100\%$$

To address reverse-causality, we further conducted sensitivity analysis by restricting the above analyses to participants after excluding past drinkers, lipid lowering agents users, the diabetics, the hypertensive, high CRP status (CRP>3mg/L), or BMI > 30 kg/m^2.

Analyses were performed using SAS 9.2 (SAS Institute, Cary, NC). Two-tailed P-value < 0.05 were considered significant.

Results

Among the 6683 participants, 55.6% were drinkers. Compared to non-drinkers, alcohol drinkers are more likely to be men, white, current smokers, and have higher education level, higher income level, higher HDL-C concentration, higher HDL-P numbers, higher apoA-Ilevel, and lower triglyceride level and lower CRP level; they are also less likely to have diabetes and hypertension (**Table 5-1**).

After an average of 8 years follow-up, 171 MI cases were documented. After adjustment of all the potential confounders, drinkers had a lower risk for MI with a nadir occurring in moderate drinkers, compared to the non-drinkers (**Table 5-2**). Adjustment of HDL-C slightly attenuated the association: the HR changed from 0.50 (95%CI: 0.25, 1.02) to 0.52 (95%CI: 0.25, 1.07) for moderate drinkers, from 0.58 (95%CI: 0.27, 1.25) to 0.62 (95%CI: 0.29, 1.34) for heavy drinkers. Adjustment of HDL-P moderately attenuated the association: the HR changed from 0.50 (95%CI: 0.25, 1.02) to 0.56 (95%CI: 0.27, 1.14), and from 0.58 (95%CI: 0.27, 1.25) to 0.69 (95%CI: 0.32, 1.51). When we added both HDL-C and HDL-P in the model, the HRs remained similar as when controlling HDL-P. In the model controlling both HDL-C and HDL-P, the HR for the HDL-C was 1.00 (95%CI: 0.99, 1.02; P=0.66), and for HDL-P was 0.97 (95%CI: 0.93, 1.00; P=0.07).

The proportion of the total effect of alcohol intake on MI that was mediated through HDL-C is 6.3% after adjustment for all the potential confounders. The mediating effect of HDL-C is not statistically significant (HR^{NIE}: 0.98; 95%CI: 0.94, 1.02; P-value=0.26) (**Table 5-3**). When examining alcohol intake classifications, results remained similar. The proportion mediated remained similar for binary alcohol intake when including the interaction term between alcohol intake and HDL-C (**Supplemental table 5-1**). Follow-up HDL-C or change rate of HDL-C as mediator remained not significant (**Supplemental table 5-2**).

The proportion of the total effect of alcohol intake on MI that was mediated through HDL-P is also slight (15.8%). Per unit change in HDL-P due to drinking alcohol versus not drinking alcohol is associated with 5% lower risk of MI, which is the mediating effect of HDL-P (HR: 0.95, 95%CI: 0.90, 0.99; P-value=0.027) (**Table 5-3**). When examining alcohol intake classifications, proportion mediated through HDL-P was 22.9% among heavy drinkers but not significant, and was 16.7% among light-to-moderate drinkers. The proportion mediated did not change substantially for binary alcohol intake when including the interaction term between alcohol intake and HDL-P (**Supplemental table 5-1**).

Sensitivity analyses showed that the mediating effect through HDL-C remained nonsignificant but HDL-P was significant in most cases (**Supplemental table 5-3**).

In the subcohort with apoA-I, adding apoA-I barely changed the HRs (**Supplemental table 5-4**). The proportion of the total effect of alcohol intake on MI that was mediated through apoA-I is 6.3% with or without accounting for alcohol- apoA-I interaction, however such mediation effect is not statistically significant (P-value=0.38 without interaction, and P-value=0.45 with interaction) (**Supplemental table 5-5**).

Discussion

In this multi-ethnic population-based prospective cohort study, we found that moderate alcohol use is related to a lower risk of MI, independent of other risk factors such as smoking, physical activity, diabetes, hypertension, BMI and total cholesterol. This protective effect was partially mediated through HDL-P but not HDL-C, or apoA-I. HDL-P explained 15.8% of the total effect of alcohol intake on risk of MI after adjustment for potential confounders including demographic, social-economic status, lifestyle behaviors and other biomarkers.

Current finding regarding the nonsignificant small mediation of HDL-C is consistent with the study that has been conducted in the Norwegian population (8), the Finish population (29) and another study conducted by us in a Chinese population, however is different from several other cohorts which reported a nearly half mediation proportion of HDL-C (1-3, 7, 30). As noted in our other study, population difference, outcome end-points, and methodology may partially explain the discrepant findings. However, using exactly same statistical approach and end-points as in the Chinese population, we found HDL-C is not significantly mediating the protective effect against CHD in this multi-ethnic population, which is composed of 40% white, 30% African-American, 20% Hispanic and 10% Asians. This finding further provided evidence for the non-central role hypothesis of HDL-C in mediating the association between alcohol and CHD irrespective of population ethnicity; it also indicated that the contrast larger mediation proportion and resulted central-role hypothesis of HDL-C suggested from previous studies could be just due to inappropriate interpretation of a large proportion number without statistical significance (1-4, 7, 30), or incomplete investigation of alcohol intake as a continuous variable or categorical variable as noted (29). Interestingly, in this study, in participants with CRP level lower than 3 mg/l, the proportion mediated by HDL-C is also about 50%, however, the indirect

effect was not statistically significant. On the other hand, such finding is not observed in the Kailuan Study.

To our knowledge, this is the first study to examine if HDL-P numbers mediate the association between alcohol intake and risk of MI. Due to lack and impossibility of long term experimental study, whether the inverse association between alcohol intake and MI is causal was challenged (30). Evidence thought to strongly support the causal relation was the observed changes in lipids, especially HDL-C, and hemostatic factors induced by alcohol intake in metabolic studies (30, 31). However, as the increased HDL-C by medical agents failed to decrease CHD in clinical trials as well as the discrepant findings about the mediating role of HDL-C, the possible mechanism of alcohol intake on CHD through HDL-C is questioned and so as the causal relation. In this current study, we found attenuation of effect estimates was driven by HDL-P with hazard ratio being 13.3%, 12% and 26.2% lower for light, moderate and heavy drinkers in a model that included HDL-P compared with models without HDL-P. For alcohol drinkers compared with non-drinkers, HDL-P mediated 15.8% of the association between alcohol and MI. Despite an observational study, using the counterfactual framework provided us an opportunity to evaluate causal interpretation of the natural direct and indirect effect. This finding provided evidence for the causal relationship between alcohol and CHD. The effect of alcohol on HDL may still be one mechanism, but it may not be through increased blood concentrations of HDL-C.

Several previous studies evaluated the relationship between the total number of HDL particles and risk of cardiovascular diseases (CVD) and found that per SD HDL-P was significant associated with 11%~32% (21, 32-34) risk reduction in CVD whereas association with HDL-C or apoA-I were weaker (33) or not significant (32, 34). This is similar to the finding from the current study, where HDL-P but not HDL-C nor apoA-1 mediated the association between alcohol intake and MI. Previously, circulating concentrations of HDL-C have been observed to be consistently

inversely associated with cardiovascular risk in observational data from different racial and ethnic groups and thus have been used as a predictor for cardiovascular risk evaluation integrated into several guidelines (15-18). However, data from human genetic studies and randomized trials have already challenged the role of HDL-C in predicting CVD(35). Introduction of new techniques has made it possible to examine other biomarkers for HDL metabolism or HDL subpopulations (36). Along with the studies mentioned above, the current study suggests that HDL-P number may be a superior factor for assessing cardiovascular risk.

As the main component of HDL, apoA-I is largely responsible for reverse cholesterol transport through the macrophage ATP-binding cassette transporter (ABCA1) and thus is thought to be a good therapeutic target (35). Small peptides that mimic some of the properties of apoA-I, have been shown in preclinical models to improve HDL function and reduce atherosclerosis without altering HDL-cholesterol levels (37), however another approach using recombinant apoA-I,-phospholipid particles was reported to have no significant effect in a coronary intravascular ultrasound trial (38). In the current study, we found neither adding apoA-I in the regression model attenuated the association between alcohol and MI nor the mediating effect of apoA-I was significant. The proportion mediated was similar to that of HDL-C. Such finding is consistent with the several aforementioned studies, in which the increase in apoA-Iconcentration probably resulted from either using gemfibrozil (32), statin and antioxidant therapy (33), or rosuvastatin (34). The reasons for these discrepant results are not well understood yet. It could be from the differences of how apoA-I was increased or from the differences of the properties of the increased apoA-I. Of note, the HDL particle number was directly elevated in the infusion studies with small peptides (37), while the mechanism of how apoA-I was increased in the latter trials was not clear.

Strength of our study include the multi-ethnic population based data with a relatively large sample size, detailed alcohol intake information, comprehensive adjustment of confounders

and the prospective cohort design enabling temporal ordering of associations between alcohol intake, HDL-C and subsequent incidences of MI. Moreover, we formally investigated mediation using a causal inference framework allowing us to decompose the total effect into natural direct and indirect effects and to quantify the proportion mediated (27, 39). We also examined mediation of HDL-C at different time points and its longitudinal change and incorporated exposure-mediator interaction in the analysis.

A number of study limitations should be acknowledged. First, some degree of misclassification of alcohol intake is likely. The self-reported data could not have been affected by the later development of MI outcome. Results from previous studies from this cohort have confirmed the validity of the alcohol data (24). Further, concentrations of HDL-C, HDL-P and apoA-I were all higher as alcohol intake increased. Second, for the results on apoA-I, because the data were only available in subset of the participants, we may not have enough power to detect a significant results even if there was a real mediation of apoA-I. Third, our conclusions are based on the assumption that there are no strong omitted confounders of the exposure-outcome and mediator-outcome associations. We have tested and adjusted for socioeconomic, lifestyle, and other potential MI risk factors, but cannot exclude that residual confounding caused by unmeasured or imprecisely measured factors could have influenced our results. However, we do not expect any potential unmeasured factors to differ largely between exposed and unexposed group and to have a large effect on MI independent of the factors that have been considered in the current study.

In conclusion, our findings suggest that the lower risk of protective effect of alcohol against CHD related to habitual alcohol intake is partially mediated through HDL-P. Although not recommended for treatment purposes, regular and light-to-moderate use of alcohol could improve CHD outcomes by increasing HDL-P. Compared with HDL-C, HDL-P appears to be a is

more promising informative biomarker for HDL metabolism and to be aa more promising therapeutic target for CHD prevention and control.

References

1. Criqui MH, Cowan LD, Tyroler HA, Bangdiwala S, Heiss G, Wallace RB, Cohn R. Lipoproteins as mediators for the effects of alcohol consumption and cigarette smoking on cardiovascular mortality: results form the Lipid Research Clinics Follow-up Study. American journal of epidemiology 1987;126:629-37.
2. Langer RD, Criqui MH, Reed DM. Lipoproteins and blood pressure as biological pathways for effect of moderate alcohol consumption on coronary heart disease. Circulation 1992;85:910-5. doi: 10.1161/01.CIR.85.3.910.
3. Suh I. Alcohol Use and Mortality from Coronary Heart Disease: The Role of High-Density Lipoprotein Cholesterol. Annals of Internal Medicine 1992;116:881. doi: 10.7326/0003-4819-116-11-881.
4. Gaziano JM, Buring JE, Breslow JL, Goldhaber SZ, Rosner B, VanDenburgh M, Willett W, Hennekens CH. Moderate Alcohol Intake, Increased Levels of High-Density Lipoprotein and Its Subfractions, and Decreased Risk of Myocardial Infarction. New England Journal of Medicine 1993;329:1829-34. doi: 10.1056/NEJM199312163292501.
5. Gaziano JM, Hennekens CH, Godfried SL, Sesso HD, Glynn RJ, Breslow JL, Buring JE. Type of alcoholic beverage and risk of myocardial infarction. The American journal of cardiology 1999;83:52-7. doi: S0002-9149(98)00782-6 [pii].
6. Djoussé L, Lee IM, Buring JE, Gaziano JM. Alcohol consumption and risk of cardiovascular disease and death in women: Potential mediating mechanisms. Circulation 2009;120:237-44. doi: 10.1161/CIRCULATIONAHA.108.832360.
7. Mukamal KJ, Jensen MK, Grønbaek M, Stampfer MJ, Manson JE, Pischon T, Rimm EB. Drinking frequency, mediating biomarkers, and risk of myocardial infarction in women and men. Circulation 2005;112:1406-13. doi: 10.1161/CIRCULATIONAHA.105.537704.
8. Magnus P, Bakke E, Hoff DA, Høiseth G, Graff-Iversen S, Knudsen GP, Myhre R, Normann PT, Næss Ø, Tambs K, et al. Controlling for high-density lipoprotein cholesterol does not affect the magnitude of the relationship between alcohol and coronary heart disease. Circulation 2011;124:2296-302. doi: 10.1161/CIRCULATIONAHA.111.036491.
9. Investigators A-H, Boden WE, Probstfield JL, Anderson T, Chaitman BR, Desvignes-Nickens P, Koprowicz K, McBride R, Teo K, Weintraub W. Niacin in Patients with Low HDL Cholesterol Levels Receiving Intensive Statin Therapy. New England Journal of Medicine 2011;365:2255-67. doi: 10.1056/NEJMoa1107579.
10. Group H-TC, Landray MJ, Haynes R, Hopewell JC, Parish S, Aung T, Tomson J, Wallendszus K, Craig M, Jiang L, et al. Effects of Extended-Release Niacin with Laropiprant in High-Risk Patients. New England Journal of Medicine 2014;371:203-12. doi: 10.1056/NEJMoa1300955.
11. Barter PJ, Caulfield M, Eriksson M, Grundy SM, Kastelein JJP, Komajda M, Lopez-Sendon J, Mosca L, Tardif J-C, Waters DD, et al. Effects of Torcetrapib in Patients at High Risk for Coronary Events. New England Journal of Medicine 2007;357:2109-22. doi: 10.1056/NEJMoa0706628.
12. Schwartz GG, Olsson AG, Abt M, Ballantyne CM, Barter PJ, Brumm J, Chaitman BR, Holme IM, Kallend D, Leiter LA, et al. Effects of Dalcetrapib in Patients with a Recent Acute Coronary Syndrome. New England Journal of Medicine 2012;367:2089-99. doi: 10.1056/NEJMoa1206797.

13. Group THTRC. Effects of Anacetrapib in Patients with Atherosclerotic Vascular Disease. New England Journal of Medicine 2017;377:1217-27. doi: 10.1056/NEJMoa1706444.
14. Lincoff AM, Nicholls SJ, Riesmeyer JS, Barter PJ, Brewer HB, Fox KAA, Gibson CM, Granger C, Menon V, Montalescot G, et al. Evacetrapib and Cardiovascular Outcomes in High-Risk Vascular Disease. New England Journal of Medicine 2017;376:1933-42. doi: 10.1056/NEJMoa1609581.
15. Castelli WP, Doyle JT, Gordon T, Hames CG, Hjortland MC, Hulley SB, Kagan A, Zukel WJ. HDL cholesterol and other lipids in coronary heart disease. The cooperative lipoprotein phenotyping study. Circulation 1977;55(5):767-72. doi: 10.1161/01.cir.55.5.767
10.1161/01.cir.55.5.767.
16. Miller NE, Thelle DS, Forde OH, Mjos OD. The Tromso heart-study. High-density lipoprotein and coronary heart-disease: a prospective case-control study. Lancet (London, England) 1977;1(8019):965-8. doi: 10.1016/s0140-6736(77)92274-7
10.1016/s0140-6736(77)92274-7.
17. Goff DC, Jr., Lloyd-Jones DM, Bennett G, Coady S, D'Agostino RB, Gibbons R, Greenland P, Lackland DT, Levy D, O'Donnell CJ, et al. 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation 2013;129(25 Suppl 2):S49-73. doi: 10.1161/01.cir.0000437741.48606.98
10.1161/01.cir.0000437741.48606.98. Epub 2013 Nov 12.
18. Toth PP, Barter PJ, Rosenson RS, Boden WE, Chapman MJ, Cuchel M, D'Agostino RB, Sr., Davidson MH, Davidson WS, Heinecke JW, et al. High-density lipoproteins: a consensus statement from the National Lipid Association. J Clin Lipidol 2013;7(5):484-525. doi: 10.1016/j.jacl.2013.08.001
10.1016/j.jacl.2013.08.001. Epub 2013 Aug 11.
19. Miller BAKMJCAKaNE. HDL-targeted therapies: progress, failures and future. Nature Review 2014;13:445.
20. Khera AV, Cuchel M, de la Llera-Moya M, Rodrigues A, Burke MF, Jafri K, French BC, Phillips JA, Mucksavage ML, Wilensky RL, et al. Cholesterol Efflux Capacity, High-Density Lipoprotein Function, and Atherosclerosis. New England Journal of Medicine 2011;364:127-35. doi: 10.1056/NEJMoa1001689.
21. MacKey RH, Greenland P, Goff DC, Lloyd-Jones D, Sibley CT, Mora S. High-density lipoprotein cholesterol and particle concentrations, carotid atherosclerosis, and coronary events: MESA (Multi-Ethnic Study of Atherosclerosis). Journal of the American College of Cardiology 2012;60:508-16. doi: 10.1016/j.jacc.2012.03.060.
22. Bild DE, Bluemke DA, Burke GL, Detrano R, Diez Roux AV, Folsom AR, Greenland P, Jacob DR, Kronmal R, Liu K, et al. Multi-Ethnic Study of Atherosclerosis: objectives and design. American journal of epidemiology 2002;156:871-81.
23. Steffen BT, Guan W, Remaley AT, Paramsothy P, Heckbert SR, McClelland RL, Greenland P, Michos ED, Tsai MY. Utility of lipoprotein particle measures for assessing coronary heart disease risk post-AHA/ACC guidelines: The Multi-Ethnic Study of Atherosclerosis. Arterioscler Thromb Vasc Biol 2015;35(2):448-54. doi: 10.1161/atvbaha.114.304349
10.1161/ATVBAHA.114.304349.
24. McClelland RL, Bild DE, Burke GL, Mukamal KJ, Lima JA, Kronmal RA. Alcohol and coronary artery calcium prevalence, incidence, and progression: Results from the Multi-Ethnic Study of Atherosclerosis (MESA). American Journal of Clinical Nutrition 2008;88:1593-601. doi: 10.3945/ajcn.2008.26420.

25. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18(6):499-502.
26. VanderWeele TJ. Causal mediation analysis with survival data. Epidemiology (Cambridge, Mass) 2011;22:582-5. doi: 10.1097/EDE.0b013e31821db37e.
27. VanderWeele T. Explanation in Causal Inference. 2015:729.
28. Vanderweele TJ, Vansteelandt S. Odds ratios for mediation analysis for a dichotomous outcome. Am J Epidemiol 2010;172(12):1339-48. doi: 10.1093/aje/kwq332.
29. Mänttäri M, Tenkanen L, Alikoski T, Manninen V. Alcohol and coronary heart disease: the roles of HDL-cholesterol and smoking. Journal of internal medicine 1997;241:157-63.
30. Rimm EB, Moats C. Alcohol and Coronary Heart Disease: Drinking Patterns and Mediators of Effect. Annals of Epidemiology 2007;17:S3-S7. doi: 10.1016/j.annepidem.2007.01.002.
31. Ronksley PE, Brien SE, Turner BJ, Mukamal KJ, Ghali Wa. Association of alcohol consumption with selected cardiovascular disease outcomes: a systematic review and meta-analysis. Bmj 2011;342:d671-d. doi: 10.1136/bmj.d671.
32. Otvos JD, Collins D, Freedman DS, Shalaurova I, Schaefer EJ, McNamara JR, Bloomfield HE, Robins SJ. Low-density lipoprotein and high-density lipoprotein particle subclasses predict coronary events and are favorably changed by gemfibrozil therapy in the Veterans Affairs High-Density Lipoprotein Intervention Trial. Circulation 2006;113(12):1556-63. doi: 10.1161/circulationaha.105.565135.
33. Parish S, Offer A, Clarke R, Hopewell JC, Hill MR, Otvos JD, Armitage J, Collins R. Lipids and lipoproteins and risk of different vascular events in the MRC/BHF Heart Protection Study. Circulation 2012;125(20):2469-78. doi: 10.1161/circulationaha.111.073684.
34. Mora S, Glynn RJ, Ridker PM. High-density lipoprotein cholesterol, size, particle number, and residual vascular risk after potent statin therapy. Circulation 2013;128(11):1189-97. doi: 10.1161/circulationaha.113.002671.
35. Rader DJ, Hovingh GK. HDL and cardiovascular disease. The Lancet 2014;384:618-25. doi: 10.1016/S0140-6736(14)61217-4.
36. Matera R, Horvath KV, Nair H, Schaefer EJ, Asztalos BF. HDL Particle Measurement: Comparison of 5 Methods. Clin Chem 2018;64(3):492-500. doi: 10.1373/clinchem.2017.277632.
37. Nissen SE, Tsunoda T, Tuzcu EM, Schoenhagen P, Cooper CJ, Yasin M, Eaton GM, Lauer MA, Sheldon WS, Grines CL, et al. Effect of recombinant ApoA-I Milano on coronary atherosclerosis in patients with acute coronary syndromes: a randomized controlled trial. Jama 2003;290(17):2292-300. doi: 10.1001/jama.290.17.2292.
38. Tardif JC, Ballantyne CM, Barter P, Dasseux JL, Fayad ZA, Guertin MC, Kastelein JJ, Keyserling C, Klepp H, Koenig W, et al. Effects of the high-density lipoprotein mimetic agent CER-001 on coronary atherosclerosis in patients with acute coronary syndromes: a randomized trial. Eur Heart J 2014;35(46):3277-86. doi: 10.1093/eurheartj/ehu171.
39. Jiang Z, VanderWeele TJ. When is the difference method conservative for assessing mediation? Am J Epidemiol 2015;182(2):105-8. doi: 10.1093/aje/kwv059.

Table 5-1. Baseline Characteristics of participants in the MESA cohort by alcohol intake (N=6683)[1-3]

		Alcohol intake				
		Non-drinkers (n=2969; 44.4%)	Light (n=2818; 42.2%)	Moderate (n=552; 8.3%)	High (n=344; 5.1%)	P-value
Men, %		39.4	53.6	51.8	56.1	<.0001
Baseline age, y		63 ± 10[2]	61 ± 10	62 ± 10	60 ± 10	<.0001
Race/Ethnicity, %	White	24.3	44.8	62.1	71.8	<.0001
	Chinese	18.4	8.2	2.5	1.5	
	Black	31.1	26.4	21.9	14.2	
	Hispanic	26.2	20.6	13.4	12.5	
Education: <high school, %		26.3	11.9	9.1	9.9	<.0001
Income: <$25,000, %		34.9	15.9	12.3	13.5	<.0001
Cigarette smoking, %	Never	59.8	47.2	30.1	25	<.0001
	Past	30.8	38.9	50.9	47.1	
	Current	9.4	13.9	19	27.9	

Total intentional exercise, MET-min		1343 ± 2186	1737 ± 2491	1742 ± 2217	1571 ± 2313	<.0001
BMI, kg/m^2		28.6 ± 5.7	28.4 ± 5.3	27.3 ± 4.7	27.4 ± 4.7	<.0001
Waist circumference, cm		98.6 ± 14.9	98.2 ±14.0	96.2 ± 13.8	97.2 ± 14.0	0.0009
HDL-C, mg/dl		49.6 ± 13.9	50.3 ± 14.3	56.7 ± 17.0	59.3 ± 18.8	<.0001
HDL-P, umol/l		33.4 ± 6.4	33.7 ± 6.3	36.8 ± 7.1	38.3 ± 7.6	<.0001
LDL-C, mg/dl		116.8 ± 31.6	118.0 ± 31.2	116.7 ± 31.4	114.7 ± 31.1	0.23
Triglyceride, mg/dl		116.7 (1.70)	111.1 (1.67)	106.7 (1.73)	111.1 (1.77)	<.0001
Total cholesterol, mg/dl		192.9 ± 35.8	193.7 ± 35.2	197.8 ± 34.1	200.2 ± 34.7	<.0001
C-reactive protein, mg/L		2.01 (3.22)	1.86 (3.16)	1.68 (3.22)	1.80 (3.13)	0.16
Apolipoprotein A_1		123.2 ± 25.2	124.5 ± 34.1	132.5 ± 28.3	137.0 ± 30.5	<.0001
DM	Normal	68.8	77.2	79.7	77.9	<.0001
	IFG	14	13	15.2	16	
	DM	17.2	9.8	5.1	6.1	
HTN	HTN	50.1	40.5	59.2	57.9	<.0001
	Normal	49.9	59.5	40.8	42.2	

[1] Participants were classified into the following categories of alcohol consumption: non-drinkers, light (women: 0-0.4 drinks/d; men: 0-0.9 drinks/d), moderate (women: 0.5-1.0 drinks/d; men: 1-2 drinks/d), and heavy (women: >1.0 drinks/d; men: > 2drinks/d).

[2] Mean ± SD (all such values unless otherwise indicated). Means were compared with the use of a general linear model, adjusted for age and sex.

[3] Values are geometric means (SD). Means were compared with the use of a general linear model, adjusted for age and sex.

Table 5-2 Adjusted hazard ratios (HRs) and 95% confidence intervals (95% CI) for risk of MI by baseline alcohol intake (N=6683)[1]

	Alcohol consumption				P for trend
	None	Light	Moderate	Heavy	
Case/N	85/2947	69/2806	9/546	8/344	
Person-years	20720	20317	3991	2511	
Incident rate/1,000 person-years	4.10	3.40	2.26	3.19	
Model 1	Ref (1)	0.74 (0.53, 1.03)	0.45 (0.22,0.90)	0.69 (0.33, 1.45)	0.025
Model 2	Ref (1)	0.78 (0.56, 1.10)	0.46 (0.23, 0.94)	0.63 (0.29, 1.33)	0.027
Model 3	Ref (1)	0.85 (0.60, 1.19)	0.50 (0.25, 1.02)	0.58 (0.27, 1.25)	0.039
Model 4	Ref (1)	0.86 (0.61, 1.21)	0.52 (0.25, 1.07)	0.62 (0.29, 1.34)	0.064
Model 5	Ref (1)	0.87 (0.62, 1.23)	0.56 (0.27, 1.14)	0.69 (0.32, 1.51)	0.12
Model 6	Ref (1)	0.87 (0.62, 1.22)	0.55 (0.27, 1.14)	0.69 (0.32, 1.50)	0.12

[1]Model 1: adjusted age, sex (man and woman), race (white, Black, Hispanic, and Chinese) and study site;

Model 2: model 1 + education level (< high school, or ≥ high school), income level (<$25000, or ≥$25000), total intentional exercise, smoking status (never, past, or current);

Model 3: model 2 + family history of MI, blood glucose status (normoglycemia, prediabetes, or diabetes), hypertension (yes or no), body mass index (<25, 25-30, 30-40, ≥40 kg/m^2), waist circumferences (men>102cm; women>88cm), C-reactive protein (<1, 1-3, >3), total cholesterol (<200, 200-239, or ≥240 mg/dl);

Model 4: model 3+ HDL-C;

Model 5: model 3+ HDL-P;

Model 6: model 3+ HDL-C, and HDL-P

Table 5-3 Natural direct and indirect effect of alcohol intake on MI and the proportion mediated through HDL-C or HDL-P in the MESA cohort (N=6683)[1-2]

	HR^{NDE} (95%CI)	P-value	HR^{NIE} (95% CI)	P-value	HR^{TE} (95%CI)	P-value	Proportion mediated
Alcohol in 2000 -> HDL-C in 2000 -> MI							
Exposure: binary alcohol intake							
Yes vs. no	0.77 (0.55, 1.07)	0.12	0.98 (0.94, 1.02)	0.26	0.75 (0.54, 1.04)	0.085	6.3%
Exposure: Alcohol intake level							
Light-to-moderate vs. no	0.77 (0.55, 1.08)	0.13	0.98 (0.94, 1.01)	0.20	0.75 (0.54, 1.05)	0.098	7.1%
Heavy vs. no	0.67 (0.30, 1.48)	0.32	0.98 (0.81, 1.18)	0.81	0.65 (0.30, 1.41)	0.28	3.9%
Alcohol in 2000 -> HDL-P in 2000 -> MI							
Exposure: binary alcohol intake							
Yes vs. no	0.79 (0.57, 1.10)	0.17	0.95 (0.90, 0.99)	0.027	0.75 (0.54, 1.04)	0.085	15.8%
Exposure: Alcohol intake amount							
Light-to-moderate versus no	0.80 (0.57, 1.12)	0.19	0.95 (0.90, 0.99)	0.013	0.75 (0.54, 1.05)	0.096	16.7%
Heavy versus no	0.73 (0.32, 1.64)	0.45	0.89 (0.72, 1.10)	0.89	0.65 (0.30, 1.42)	0.28	22.9%

[1] NDE: natural direct effect; NIE: natural indirect effect; TE: total effect

[2] All models adjusted age, sex (man and woman), race (white, Black, Hispanic, and Chinese), study site, education level (< high school, or ⩾ high school), income level (<$25000, or ⩾$25000 per year), total intentional exercise, smoking status (never, past, or current), blood glucose status (normoglycemia, prediabetes, or diabetes), hypertension (yes or no), body mass index (<25, 25-30, 30-40, or ⩾40 kg/m^2), waist circumferences (men>102cm; women>88cm), C-reactive protein (<1, 1-3, or >3mg/l), and total cholesterol (<200, 200-239, or ⩾240 mg/dl).

Legends for figures

Figure 5-1: Flowchart of the study

Figure 5-2: Mediation analysis of the effects of HDL-C concentration on the association between alcohol consumption and MI

Figure 5-1 Flowchart of the study

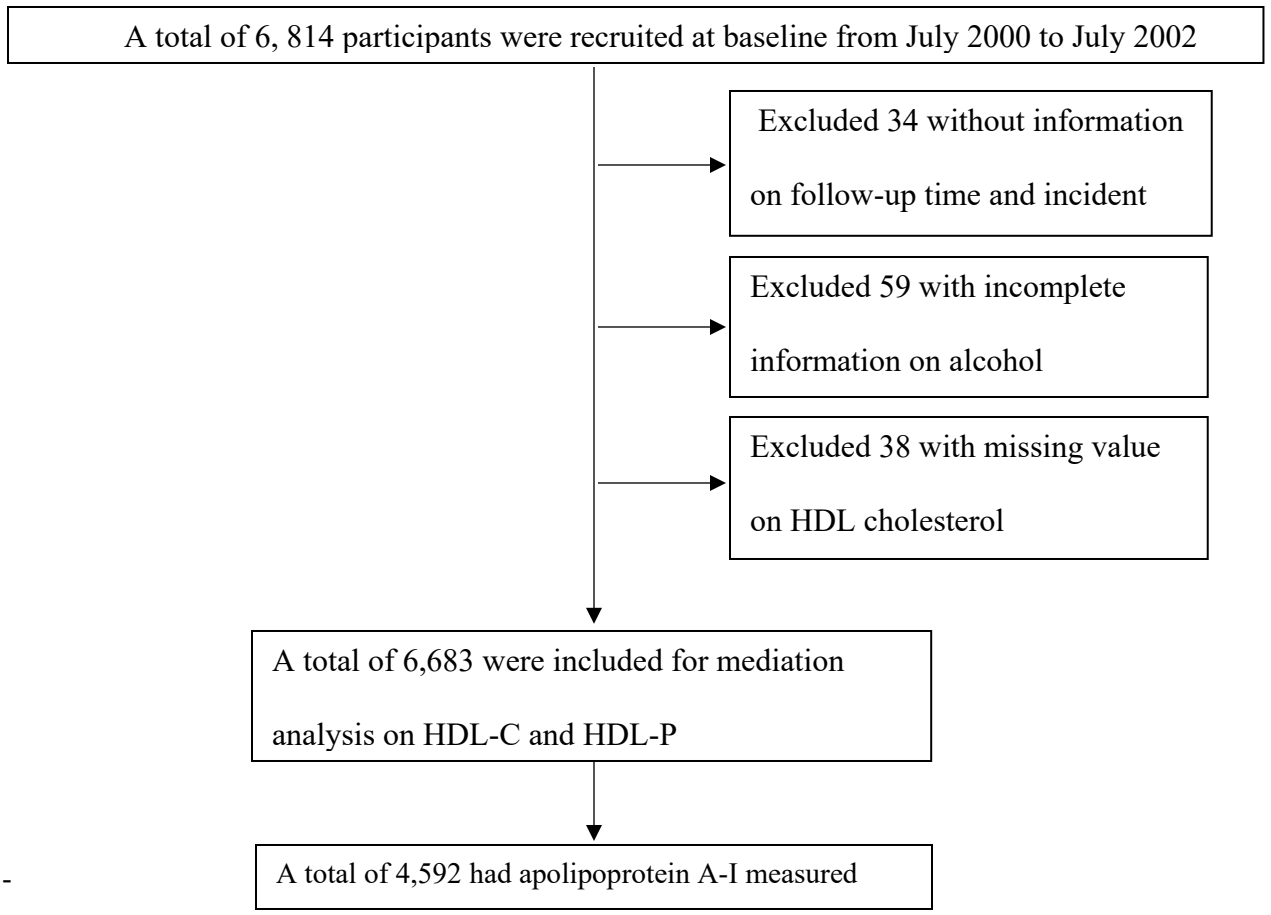
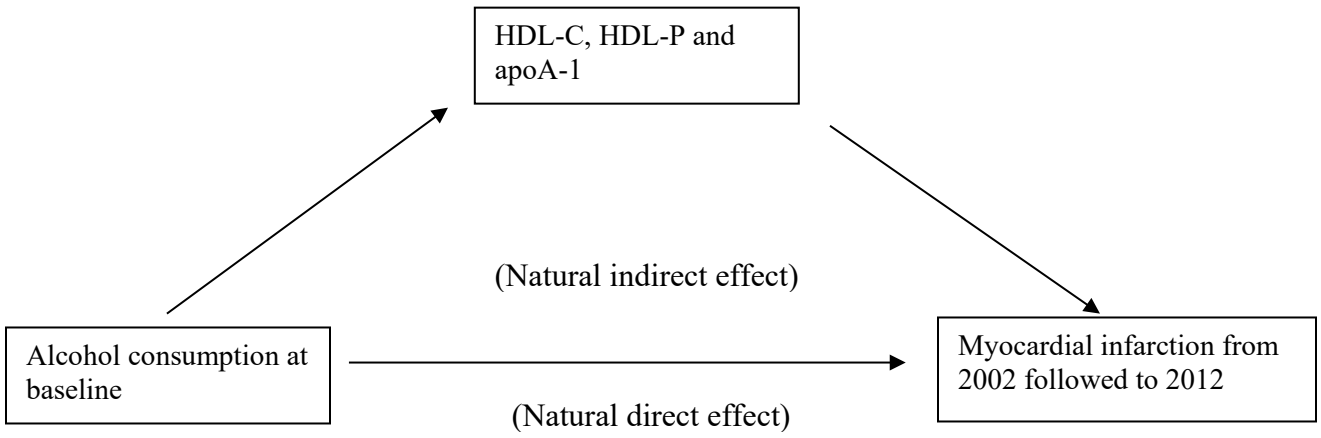


Figure 5-2 HDL cholesterol mediates the association between alcohol consumption and risk of myocardial infarction



Supplemental table 5-1 Natural direct and indirect effect of alcohol intake on MI and the proportion mediated through HDL-C or HDL-P accounting for alcohol-mediator interaction in the MESA cohort (N=6683)[1-2]

	HR^{NDE} (95%CI)	P-value	HR^{NIE} (95% CI)	P-value	HR^{TE} (95%CI)	P-value	Proportion mediated
Alcohol in 2000 -> HDL-C in 2000 -> MI							
Exposure: binary alcohol intake							
Yes vs. no	0.76 (0.55, 1.07)	0.11	0.97 (0.92, 1.02)	0.30	0.74 (0.53, 1.04)	0.082	8.7%
Exposure: Alcohol intake level							
Light-to-moderate vs. no	0.77 (0.55, 1.08)	0.12	0.97 (0.92, 1.02)	0.21	0.74 (0.53, 1.04)	0.088	9.1%
Heavy vs. no	0.61 (0.26, 1.42)	0.25	1.15 (0.75, 1.77)	0.51	0.71 (0.32, 1.58)	0.40	-30.7%
Alcohol in 2000 -> HDL-P in 2000 -> MI							
Exposure: binary alcohol intake							
Yes vs. no	0.79 (0.57, 1.10)	0.17	0.94 (0.88, 1.00)	0.065	0.75 (0.54, 1.04)	0.083	18.4%
Exposure: Alcohol intake amount							
Light-to-moderate versus no	0.80 (0.57, 1.11)	0.18	0.93 (0.88, 0.99)	0.023	0.74 (0.53, 1.04)	0.082	21.9%
Heavy versus no	0.53 (0.21, 1.35)	0.18	1.36 (0.78, 2.36)	0.27	0.73 (0.33, 1.60)	0.43	-68.3%

[1]NDE: natural direct effect; NIE: natural indirect effect; TE: total effect

[2]All models adjusted age, sex (man and woman), race (white, Black, Hispanic, and Chinese), study site, education level (< high school, or ⩾ high school), income level (<$25000, or ⩾$25000), total intentional exercise, smoking status (never, past, or current), blood glucose status (normoglycemia, prediabetes, or diabetes), hypertension (yes or no), body mass index (<25, 25-30, 30-40, ⩾40 kg/m^2), waist circumferences (men>102cm; women>88cm), C-reactive protein (<1, 1-3, >3 mg/l), and total cholesterol (<200, 200-239, or ⩾240 mg/dl).

Supplemental table 5-2 Natural direct and indirect effects of alcohol intake on MI and the proportion mediated through HDL-C during follow-up and change in HDL-C in the MESA cohort[1-3]

	Case/N	HR^{NDE} (95% CI)	P-value	HR^{NIE} (95% CI)	P-value	HR^{TE} (95% CI)	P-value	Proportion mediated
HDL-C at Survey 2 (2003)	151/6103	0.73 (0.49, 1.07)	0.11	0.995 (0.98,1.01)	0.45	0.72 (0.49, 1.07)	0.10	1.3%
HDL-C at Survey 3 (2005)	96/5816	0.69 (0.43, 1.09)	0.12	0.994 (0.98, 1.01)	0.42	0.69 (0.43, 1.09)	0.11	1.3%
HDL-C at Survey 4 (2007)	57/5562	1.09 (0.59, 2.02)	0.78	1.00 (0.99, 1.01)	0.58	1.09 (0.59, 2.03)	0.78	3.5%
Change rate in HDL-C	158/6280	0.80 (0.56, 1.13)	0.20	0.996 (0.98, 1.01)	0.45	0.79 (0.56, 1.13)	0.20	1.6%

[1] NDE, natural direct effect; NIE, natural indirect effect; HR hazard ratio; TE, total effect

[2] Hazard ratios for the effect of alcohol intake yes vs no (reference group).

A causal inference framework for mediation analysis was used to estimate HR and 95% CI for total, natural direct and indirect effects. The natural direct and indirect effects were estimated by fitting a Cox regression model for the time-to-event outcome, and a linear regression

model for the continuous mediator. From these combined models HR of natural direct and indirect effects were derived. Total effects are equal to the product of the natural direct and indirect effects.

The proportion mediated was calculated as $[HR^{NDE} (HR^{NIE} – 1)] / [HR^{NDE} \times HR^{NIE} – 1] \times 100\%$, and approximates the extent to which the effect of the alcohol on time-to-MI is mediated through HDL-C relative to the overall effect of the alcohol.

Alcohol-HDL-C interaction was accounted for in the model.

[3] Adjusted for age, sex, race, study site, educational level, income level, exercise, smoking status, blood glucose status (normoglycemia, prediabetes, or diabetes), hypertension (yes or no), body mass index (<25, 25-30, 30-40, ≥40 kg/m^2), waist circumferences (men>102cm; women>88cm), C-reactive protein (<1, 1-3, >3mg/l), total cholesterol (<200, 200-239, or ≥240 mg/dl), and baseline HDL-C.

Supplemental table 5-3 Sensitivity analysis on natural direct and indirect effect of alcohol intake on MI and the proportion mediated through HDL-C or HDL-P (N=6683)[1-3]

	HR^{NDE} (95%CI)	P-value	HR^{NIE} (95% CI)	P-value	HR^{TE} (95%CI)	P-value	Proportion mediated
Alcohol in 2000 -> HDL-C in 2000 -> MI							
Exposure: binary alcohol intake							
Excluding past drinkers	0.75 (0.45, 1.23)	0.26	0.97 (0.92, 1.02)	0.29	0.73 (0.44, 1.19)	0.21	8.3%
Excluding lipid lowering	0.77 (0.53, 1.12)	0.16	0.97 (0.91,1.03)	0.3	0.74 (0.51, 1.08)	0.12	9.1%
Excluding diabetes	0.71 (0.49, 1.04)	0.08	0.97 (0.91, 1.02)	0.25	0.69 (0.47, 1.00)	0.05	6.8%
Excluding hypertension	0.60 (0.35, 1.03)	0.037	0.996 (0.92, 1.08)	0.93	0.60 (0.35, 1.03)	0.064	0.6%
Excluding CRP > 3 mg/L	0.98 (0.64, 1.49)	0.92	0.97 (0.90, 1.04)	0.41	0.95 (0.62, 1.45)	0.8	59.5%
Excluding BMI > 30 kg/m^2	0.84 (0.56, 1.26)	0.43	0.97 (0.90, 1.04)	0.36	0.82 (0.55, 1.22)	0.32	13.6%
Alcohol in 2000 -> HDL-P in 2000 -> MI							
Exposure: binary alcohol intake							
Excluding past drinkers	0.77 (0.47, 1.27)	0.31	0.95 (0.89, 1.00)	0.059	0.73 (0.44, 1.20)	0.22	14.3%
Excluding lipid lowering	0.82 (0.56, 1.19)	0.29	0.88 (0.81, 0.95)	0.0018	0.72 (0.49, 1.04)	0.081	35.3%

Excluding diabetes	0.76 (0.52, 1.10)	0.15	0.92 (0.86, 0.99)	0.029	0.70 (0.48, 1.01)	0.058	20.2%
Excluding hypertension	0.58 (0.34, 0.99)	0.047	0.96 (0.88, 1.06)	0.42	0.56 (0.32, 0.96)	0.036	5.2%
Excluding CRP > 3 mg/L	1.02 (0.67, 1.56)	0.92	0.93 (0.85, 1.01)	0.096	0.95 (0.62, 1.45)	0.81	138.9%
Excluding BMI > 30 kg/m^2	0.88 (0.59, 1.32)	0.54	0.93 (0.86, 1.01)	0.068	0.82 (0.55, 1.22)	0.33	33.9%

[1] NDE, natural direct effect; NIE, natural indirect effect; HR hazard ratio; TE, total effect

[2] Hazard ratios for the effect of alcohol intake yes vs no (reference group).

A causal inference framework for mediation analysis was used to estimate HR and 95% CI for total, natural direct and indirect effects. The natural direct and indirect effects were estimated by fitting a Cox regression model for the time-to-event outcome, and a linear regression model for the continuous mediator. From these combined models HR of natural direct and indirect effects were derived. Total effects are equal to the product of the natural direct and indirect effects.

The proportion mediated was calculated as $[HR^{NDE} (HR^{NIE} – 1)] / [HR^{NDE} \times HR^{NIE} – 1] \times 100\%$, and approximates the extent to which the effect of the alcohol on time-to-MI is mediated through HDL-C relative to the overall effect of the alcohol.

Alcohol-HDL-C interaction was accounted for in the model.

[3] Adjusted for age, sex, race, study site, educational level, income level, exercise, smoking status, blood glucose status (normoglycemia, prediabetes, or diabetes), hypertension (yes or no), body mass index (<25, 25-30, 30-40, ≥40 kg/m^2), waist circumferences (men>102cm; women>88cm), C-reactive protein (<1, 1-3, >3mg/l), and total cholesterol (<200, 200-239, or ≥240 mg/dl).

Supplemental table 5-4 Adjusted hazard ratios (HRs) and 95% confidence intervals (95% CI) for risk of MI by baseline alcohol intake among participants with apoA-Ⅰ (N=4592)

	Alcohol consumption				P for
	None	Light	Moderate	Heavy	trend
Case/N	51/2064	42/1909	5/377	3/242	
Person-years	11463	13790	2761	1769	
Incident rate/1,000 person-years	0.4%	0.3%	0.2%	0.2%	
Model 1	Ref (1)	0.82 (0.53, 1.25)	0.42 (0.17, 1.08)	0.44 (0.13, 1.43)	0.034
Model 2	Ref (1)	0.83 (0.54, 1.29)	0.44 (0.17, 1.13)	0.41 (0.12, 1.36)	0.038
Model 3	Ref (1)	0.86 (0.56, 1.34)	0.47 (0.18, 1.20)	0.39 (0.12, 1.28)	0.043
Model 4	Ref (1)	0.87 (0.56, 1.36)	0.48 (0.19, 1.24)	0.40 (0.12, 1.34)	0.055

Model 1: adjusted age, sex (man and woman), race (white, Black, Hispanic, and Chinese) and site;

Model 2: model 1 + education level (< high school, or ≥ high school), income level (<$25000, or ≥$25000), total intentional exercise, smoking status (never, past, or current);

Model 3: model 2 + blood glucose status (normoglycemia, prediabetes, or diabetes), hypertension (yes or no), body mass index (<25, 25-30, 30-40, ≥40 kg/m^2), waist circumferences (men>102cm; women>88cm), C-reactive protein (<1, 1-3, >3mg/l), total cholesterol (<200, 200-239, or ≥240 mg/dl);

Model 4: model 3+ apoA-I.

Supplemental table 5-5 Natural direct and indirect effect of alcohol intake on MI and the proportion mediated through apoA-Ⅰ with and without accounting for alcohol-apoA-Ⅰ interaction[1](N=4592)

	HR^{NDE} (95%CI)	P-value	HR^{NIE} (95% CI)	P-value	HR^{TE} (95%CI)	P-value	Proportion mediated
Alcohol in 2000 -> apoA-I in 2000 -> MI[2]							
Exposure: binary alcohol intake							
Yes vs. no	0.77 (0.50, 1.18)	0.22	0.98 (0.93, 1.03)	0.38	0.75 (0.49, 1.15)	0.19	6.3%
Exposure: Alcohol intake level							
Light-to-moderate vs. no	0.79 (0.51, 1.21)	0.28	0.98 (0.94, 1.02)	0.31	0.77 (0.50, 1.18)	0.23	7.0%
Heavy vs. no	0.40 (0.11, 1.39)	0.15	1.04 (0.85, 1.26)	0.73	0.41 (0.12, 1.41)	0.16	-2.7%
Alcohol in 2000 -> apoA-I in 2000 -> MI[3]							
Exposure: binary alcohol intake							
Yes vs. no	0.77 (0.50, 1.18)	0.22	0.98 (0.92, 1.04)	0.45	0.75 (0.49, 1.15)	0.18	6.3%
Exposure: Alcohol intake level							
Light-to-moderate vs. no	0.79 (0.51, 1.22)	0.28	0.97 (0.91, 1.03)	0.30	0.77 (0.50, 1.18)	0.22	10.1%
Heavy vs. no	0.29 (0.074, 1.15)	0.078	1.56 (0.90, 2.72)	0.11	0.45 (0.13, 1.57)	0.21	-29.7%

[1]All models adjusted age, sex (man and woman), race (white, Black, Hispanic, and Chinese), study site, education level (< high school, or ⩾ high school), income level (<$25000, or ⩾$25000), total intentional exercise, smoking status (never, past, or current), blood glucose status (normoglycemia, prediabetes, or diabetes), hypertension (yes or no), body mass index (<25, 25-30, 30-40, ⩾40 kg/m^2), waist circumferences (men>102cm; women>88cm), C-reactive protein (<1, 1-3, >3mg/l), and total cholesterol (<200, 200-239, or ⩾240 mg/dl).

[2] Model without alcohol-apoA-I interaction

[3]Model with alcohol-apoA-I interaction

Chapter 6

Conclusions

Summary of research findings and implications

The studies in this dissertation research project were designed to further understand the interrelationship among alcohol, HDL and MI risk. These studies 1) examined the association between baseline alcohol intake and cross-sectional HDL-C concentration and longitudinal change of HDL-C concentrations; 2) investigated the mediating effects of HDL-C concentration for alcohol intake and MI risk; and 3) further examined other HDL metabolism biomarkers including HDL-P and apoA-I in mediating the effects of alcohol intake on MI risk.

Chapter 3 addressed objective 1, examining the association between baseline alcohol intake and cross-sectional and longitudinal change of HDL-C concentrations. We found a linear dose-response association between greater alcohol consumption and higher baseline HDL-C concentrations. We also found an umbrella-shaped association between total alcohol consumption and time-dependent change in HDL-C concentrations, with a lowest decrease observed in moderate alcohol drinkers. We also examined the impact of different type of alcoholic beverage on change in lipids and found moderate consumption of hard liquor was associated with a greater effect on HDL-C and lipid ratios, whereas higher intake of beer was associated with an improved lipid profiles.

In this study, moderate alcohol consumption was related to an average lower decrease in HDL-C concentration of 0.017mmol/l per year (95%CI 0.009-0.025mmol/l), which amounted to 0.102 mmol/l over the 6 years' follow-up. Based on previous data, this would be associated with a risk reduction in CVD of approximately 13.1% (1). There are several potential pathways for the

protective effect of alcohol on CVD through its impact on HDL. One aspect is that alcohol can raise HDL subfractions including both HDL_2 and HDL_3 (2, 3)as well as major HDL lipoproteins, including apolipoprotein A-I and apo A–II (3). The inverse relation between the HDL_2 subfraction and the risk of MI has been clearly demonstated whereas the data on the role of HDL_3 are less consistent (4). Studies have also suggested that the prominent effect of alcohol on HDL turnover is an increase in apoA-I transporting rates (5). Alcohol may also increase both the total phospholipid (PL) and polyunsaturated fatty acid content of HDL (6), which may increase the fluidity of HDL. Further, alcohol promotes the conversion of phosphatidylcholine to phosphstidylethanol in HDL, which increases HDL binding to endothelial cells and up-regulates vascular endothelial growth factor, protecting against atherosclerosis (7). However, long-term heavy alcohol consumption may develop severe liver diseases, in which case the liver's production of HDL would decrease (8).

Chapter 4 addressed objective 2, investigating the mediating role of HDL-C in the relation between alcohol intake and MI in the Kailuan cohort. Greater alcohol intake was found to be related to a lower risk of MI in the Chinese adults, independent of other risk factors for heart disease such as smoking, physical activity, BMI, diabetes, blood pressure, and total cholesterol. Compared to non-drinkers, drinkers had a 34% lower risk for MI (HR=0.66, 95%CI: 0.56, 0.78). The association between alcohol intake and MI did not change substantially when adding HDL-C into the Cox regression model. A non-significant slight proportion (~2%) was observed for HDL-C in mediating the association between alcohol intake and MI risk. Sex, age, and cardiovascular risk did not modify the main results. Excluding past drinkers, participants using lipid-lowering agents, with diabetes, with hypertension, with higher inflammation status of higher BMI, or coal miners did not change our results substantially. Therefore, the protective effect of alcohol on MI may not be through HDL-C. Experimental or metabolic studies have demonstrated that several other biological markers associated with CHD, including other lipids, hemostatic factors such as

fibrinogen, inflammatory status, and glycemic control parameter could be improved by light-to-moderate alcohol intake (3). These factors could be potential mediators for the relation between alcohol intake and MI. However, whether or to what extent they are mediating should be further investigated in future studies.

Given the controversial data generated from human genetic data regarding HDL-C concentrations (9-11) and failed clinical trials using HDL-C raising agents to reduce CVD risk (12, 13), along with the finding from this study, it is well shown that HDL-C raised by alcohol intake does not necessarily lead to a protective effect for CVD. However, because alcohol raises HDL-C through multiple pathways as mentioned above (3, 14), and the composition and structure of the HDL particle is complex (15, 16), further examination is needed to test if other alcohol raised biomarkers of HDL metabolism may result in a lower risk of MI. This further motivated the third study in this dissertation.

Chapter 5 addressed objective 3, in which we used mediation analysis to investigate if in addition to HDL-C concentration, plasma concentrations of HDL-P or apoA-I mediated the relationship between alcohol intake and MI in a multi-ethnic population. Similarly, light-to-moderate alcohol intake was associated with a lower risk of MI. Such association was independent of smoking, physical activity, BMI, diabetes, blood pressure, and total cholesterol. The association between alcohol intake and MI was slightly attenuated by adjustment of HDL-C, or apoA-I concentration in the Cox model, and attenuated further by adjustment of HDL-P concentration. Mediation analysis indicated a very moderate but significant indirect effect of HDL-P. The proportion of the total effect of alcohol intake on MI mediated by HDL-P was 15.8%. Incorporating alcohol and mediator interaction, sensitivity analysis as similarly conducted in the Kailuan study did not materially change the results.

Findings from this study supported the possible causal relationship between alcohol intake and MI (17), and the pathway through HDL, despite that the mediation proportion is

relatively small and it is not through blood concentrations of HDL-C. This finding also suggests that HDL-P number may be an important factor for assessing cardiovascular risk. Both HDL-P (18-20) and apoA-I (16, 21) have been shown in intervention studies to predict reduced risk of CVD, although the evidence on apoA-I is less consistent. The discrepant finding regarding a significant mediating effect of HDL-P and a non-significant mediating role of apoA-I indicates that some properties which may 'truly' represent the HDL functionality reflected by HDL-P may not be well captured by apoA-I either. However, due to the smaller sample size regarding apoA-I, future studies should be warranted to further investigate the definitive role of apoA-I in predicting CVD.

Strengths and Limitations

The current study is, to our knowledge, the first to use a causal inference framework to formally investigate the extent to which HDL contributed to the association between alcohol intake and MI risk. Previous studies (17, 22-29) on this topic have not formally distinguished between HDL-C as a confounder or mediator in the models, and did not provide an estimate for indirect effect through HDL-C and inferential test for the estimate. In the current study, we used the counterfactual frame and formally defined direct and indirect effect of alcohol intake on MI risk and provided both P-values and 95% CI for the indirect effect, i.e. the mediating effect of HDL-C, contributing a robust evidence demonstrating HDL-C is barely mediating the protective effect of alcohol against CHD. Using this approach, we were also able to overcome some limitations of the traditional regression approach such as no interaction allowed. We examined the exposure-mediator, i.e. alcohol-HDL-C interaction in our analysis. We also examined alcohol intake both as continuous variables, binary and categorized variable in the mediation analysis.

This is also the first study to examine whether HDL-P numbers mediate the association between alcohol intake and risk of MI using mediation analysis under a causal inference framework. Previous studies have examined the predicting ability of HDL-P and HDL-C by adding both in the same regression model. However, whether the increase of HDL-P and HDL-C was resulted from alcohol intake and whether that increase can be translated into a protective effect was not investigated. In the current study, we examined both HDL-P and HDL-C as mediators for the association between alcohol intake and MI risk. Our findings suggest alcohol should not be recommended for “good” cholesterol improvement for heart disease prevention purpose and HDL cholesterol as a biomarker for HDL metabolism is not adequate. These findings contribute to the evidence for dietary recommendation on alcohol intake and also for cardiovascular prevention intervention.

With the availability of the repeated measurements of HDL-C from both studies, we were also able to examine the long-term effect of total alcohol consumption on HDL-C concentrations, and to perform the mediation using time-varying HDL-C as mediator, both of which were rarely done in previous studies. Using change of HDL-C or the HDL-C concentration during follow-up as mediator provides a temporal relation between alcohol, HDL and MI, and therefore a stronger evidence for inferring causal relation.

Our study is based on two different cohorts. These two cohorts are different in their study design, with MESA enrolled middle aged participants with some risk for cardiovascular disease from six different communities using multiple stage sampling process, and Kailuan enrolled all employees from the Kailuan coal mining company. Participants are different in many aspects such as age and sex distribution, other socioeconomic status such as education, income, and occupation, and lifestyle behaviors. Despite such difference, we found consistent results from those two populations, which provide a strong evidence for the non-significant mediating role of HDL-C between alcohol and MI and enhanced the generalizability of our finding. Other strengths of our study include a large sample size, detailed alcohol intake information, and comprehensive adjustment of confounders and the prospective cohort design enabling temporal ordering of associations between alcohol intake, HDL-C and subsequent incidences of MI.

A number of study limitations should be acknowledged. First, alcohol consumption was self-reported, and therefore some degree of misclassification of alcohol intake is likely. However, the self-reported data could not have been affected by the later development of MI outcome. Results from previous studies from both cohorts have confirmed the validity of the alcohol data (30). Further, concentrations of HDL-C, HDL-P and apoA-I were all higher as alcohol intake increased. Due to the change of questionnaire in the Kailuan cohort, we were not able to examine if alcohol intake changed during follow-up in this cohort. Second, for the results on apoA-I , because the data were only available in subset of the participants, we may not have enough power

to detect a significant results even if there was a real mediation of apoA-I . Third, our conclusions are based on the assumption that there are no strong omitted confounders of the exposure-outcome and mediator-outcome associations. We have tested and adjusted for socioeconomic, lifestyle, and other potential MI risk factors, but cannot exclude that residual confounding caused by unmeasured or imprecisely measured factors could have influenced our results. For example, in the Kailuan cohort, For example, we did not collect comprehensive dietary intake information. Because moderate drinkers might follow a healthy diet pattern, which is associated with better lipid profiles, we might have overestimated the association between alcohol intake and change in HDL-C concentrations overtime. Of note, we adjusted for factors such as BMI and waist circumference reflecting dietary intake to some extent. Regardless, we do not expect any potential unmeasured factors to differ largely between exposed and unexposed group and to have a large effect on MI independent of the factors that have been considered in the current study.

Directions for future research

There are several areas of studies that would help to promote further understanding of the interrelation among alcohol, HDL and MI risk, and provide evidence for dietary recommendation regarding alcohol intake, and also for developing effective interventions for CVD prevention:

1. It is well established that light-to-moderate alcohol consumption is related to a lower risk of CVD, and it probably does so by improving lipids profile, hemostatic factors, and inflammatory status. Studies investigate the mediating role of other possible pathways would help understand the underlying mechanism.

2. Advice regarding light-to-moderate drinking is often held because of the danger uncontrolled drinking. Reliable predictors of risk progression to heavy drinking could help provide timely personalized advice.

3. Experimental studies on the effect of alcohol intake on HDL-P concentration, HDL functionality and other subclasses such as different size of HDL particle could confirm the causal role of HDL in mediating the relationship for alcohol and CVD.

4. Although the population used to examine the predicting role of HDL-P and apoA-I is diverse in ethnicity, geographical location, and socioeconomic status, it would be important to examine whether such results could be replicated in other population from a different study design setting. Especially for apoA-I, future study with a larger sample size should be warranted.

5. The relative importance of different biological activities of HDL for atheroprotection is not fully understood. Proper evaluation of HDL function is still under investigation. Although only limited information on all aspects of circulating HDL pool can be provided, measurement of any individual HDL activity, e.g. examination of the association of HDL subclasses such as HDL_2 and HDL_3 with CVD, would contribute to the understanding of HDL function.

6. It is also valuable to investigate how alcohol intake is related to risk of stroke because both stroke and MI are pathologically due to atherosclerosis but stroke is more affected by hypertension which can be increased by alcohol, and therefore the mediation effect of HDL under such condition.

In conclusion, findings presented in this dissertation contribute to the understanding of the role of alcohol intake and HDL in cardiovascular disease. Our results have further substantiated the role of HDL-C between alcohol and CVD in two populations different in predisposition genetic features, geographical location, lifestyle behaviors, and disease spectrum. Conducting the analysis using the exact same approach, we were able to minimize the possible difference in methodology and therefore provide a strong evidence for the results. Although alcohol intake was both cross-sectionally and longitudinally associated with HDL-C, non-significant finding of the mediating effect of HDL-C from both cohorts suggest that HDL-C does

not mediate the relation between alcohol and MI but HDL-P concentrations may. Future studies should confirm our finding regarding HDL-P and further evaluate if HDL-P concentration may provide a surrogate biomarker reflecting the anti-atherogenic HDL function and to determine if HDL-P can be used as a new risk predictor and intervention target for CVD.

References

1. Stampfer MJ, Sacks FM, Salvini S, Willett WC, Hennekens CH. A prospective study of cholesterol, apolipoproteins, and the risk of myocardial infarction. N Engl J Med 1991;325(6):373-81. doi: 10.1056/nejm199108083250601.
2. Gaziano JM, Buring JE, Breslow JL, Goldhaber SZ, Rosner B, VanDenburgh M, Willett W, Hennekens CH. Moderate alcohol intake, increased levels of high-density lipoprotein and its subfractions, and decreased risk of myocardial infarction. N Engl J Med 1993;329(25):1829-34. doi: 10.1056/NEJM199312163292501.
3. Brien SE, Ronksley PE, Turner BJ, Mukamal KJ, Ghali WA. Effect of alcohol consumption on biological markers associated with risk of coronary heart disease: systematic review and meta-analysis of interventional studies. BMJ : British Medical Journal 2011;342:d636. doi: 10.1136/bmj.d636.
4. Gofman JW, Young W, Tandy R. Ischemic heart disease, atherosclerosis, and longevity. Circulation 1966;34:679-97.
5. De Oliveira E Silva ER, Foster D, McGee Harper M, Seidman CE, Smith JD, Breslow JL, Brinton Ea. Alcohol consumption raises HDL cholesterol levels by increasing the transport rate of apolipoproteins A-I and A-II. Circulation 2000;102:2347-52. doi: 10.1161/01.CIR.102.19.2347.
6. Brinton EA. Effects of ethanol intake on lipoproteins. Curr Atheroscler Rep 2012;14(2):108-14. doi: 10.1007/s11883-012-0230-7
10.1007/s11883-012-0230-7.
7. Liisanantti MK, Savolainen MJ. Phosphatidylethanol mediates its effects on the vascular endothelial growth factor via HDL receptor in endothelial cells. Alcohol Clin Exp Res 2008;33(2):283-8. doi: 10.1111/j.1530-0277.2008.00831.x
10.1111/j.1530-0277.2008.00831.x. Epub 2008 Nov 19.
8. Devenyi P, Robinson GM, Kapur BM, ... High-density lipoprotein cholesterol in male alcoholics with and without severe liver disease. The American journal of Medicine 1981;71:589-94.
9. Thompson A, Di Angelantonio E, Sarwar N, Erqou S, Saleheen D, Dullaart RP, Keavney B, Ye Z, Danesh J. Association of cholesteryl ester transfer protein genotypes with CETP mass and activity, lipid levels, and coronary risk. Jama 2008;299(23):2777-88. doi: 10.1001/jama.299.23.2777
10.1001/jama.299.23.2777.
10. Li YY, Wu XY, Xu J, Qian Y, Zhou CW, Wang B. Apo A5 -1131T/C, FgB -455G/A, -148C/T, and CETP TaqIB gene polymorphisms and coronary artery disease in the Chinese population: a meta-analysis of 15,055 subjects. Mol Biol Rep 2012;40(2):1997-2014. doi: 10.1007/s11033-012-2257-9
10.1007/s11033-012-2257-9. Epub 2012 Nov 6.
11. Oldoni F, Sinke RJ, Kuivenhoven JA. Mendelian disorders of high-density lipoprotein metabolism. Circ Res 2014;114(1):124-42. doi: 10.1161/circresaha.113.300634
10.1161/CIRCRESAHA.113.300634.
12. Investigators A-H, Boden WE, Probstfield JL, Anderson T, Chaitman BR, Desvignes-Nickens P, Koprowicz K, McBride R, Teo K, Weintraub W. Niacin in Patients with Low HDL Cholesterol Levels Receiving Intensive Statin Therapy. New England Journal of Medicine 2011;365:2255-67. doi: 10.1056/NEJMoa1107579.
13. Group H-TC, Landray MJ, Haynes R, Hopewell JC, Parish S, Aung T, Tomson J, Wallendszus K, Craig M, Jiang L, et al. Effects of Extended-Release Niacin with

Laropiprant in High-Risk Patients. New England Journal of Medicine 2014;371:203-12. doi: 10.1056/NEJMoa1300955.
14. Rimm EB, Williams P, Fosher K, Criqui M, Stampfer MJ. Moderate alcohol intake and lower risk of coronary heart disease: Meta-analysis of effects on lipids and haemostatic factors. British Medical Journal 1999;319:1523-8.
15. Miller BAKMJCAKaNE. HDL-targeted therapies: progress, failures and future. Nature Review 2014;13:445.
16. Rader DJ, Hovingh GK. HDL and cardiovascular disease. The Lancet 2014;384:618-25. doi: 10.1016/S0140-6736(14)61217-4.
17. Rimm EB, Moats C. Alcohol and Coronary Heart Disease: Drinking Patterns and Mediators of Effect. Annals of Epidemiology 2007;17:S3-S7. doi: 10.1016/j.annepidem.2007.01.002.
18. Otvos JD, Collins D, Freedman DS, Shalaurova I, Schaefer EJ, McNamara JR, Bloomfield HE, Robins SJ. Low-density lipoprotein and high-density lipoprotein particle subclasses predict coronary events and are favorably changed by gemfibrozil therapy in the Veterans Affairs High-Density Lipoprotein Intervention Trial. Circulation 2006;113(12):1556-63. doi: 10.1161/circulationaha.105.565135.
19. Parish S, Offer A, Clarke R, Hopewell JC, Hill MR, Otvos JD, Armitage J, Collins R. Lipids and lipoproteins and risk of different vascular events in the MRC/BHF Heart Protection Study. Circulation 2012;125(20):2469-78. doi: 10.1161/circulationaha.111.073684.
20. Mora S, Glynn RJ, Ridker PM. High-density lipoprotein cholesterol, size, particle number, and residual vascular risk after potent statin therapy. Circulation 2013;128(11):1189-97. doi: 10.1161/circulationaha.113.002671.
21. Nissen SE, Tsunoda T, Tuzcu EM, Schoenhagen P, Cooper CJ, Yasin M, Eaton GM, Lauer MA, Sheldon WS, Grines CL, et al. Effect of recombinant ApoA-I Milano on coronary atherosclerosis in patients with acute coronary syndromes: a randomized controlled trial. Jama 2003;290(17):2292-300. doi: 10.1001/jama.290.17.2292.
22. Criqui MH, Cowan LD, Tyroler HA, Bangdiwala S, Heiss G, Wallace RB, Cohn R. Lipoproteins as mediators for the effects of alcohol consumption and cigarette smoking on cardiovascular mortality: results form the Lipid Research Clinics Follow-up Study. American journal of epidemiology 1987;126:629-37.
23. Langer RD, Criqui MH, Reed DM. Lipoproteins and blood pressure as biological pathways for effect of moderate alcohol consumption on coronary heart disease. Circulation 1992;85:910-5. doi: 10.1161/01.CIR.85.3.910.
24. Suh I. Alcohol Use and Mortality from Coronary Heart Disease: The Role of High-Density Lipoprotein Cholesterol. Annals of Internal Medicine 1992;116:881. doi: 10.7326/0003-4819-116-11-881.
25. Gaziano JM, Buring JE, Breslow JL, Goldhaber SZ, Rosner B, VanDenburgh M, Willett W, Hennekens CH. Moderate Alcohol Intake, Increased Levels of High-Density Lipoprotein and Its Subfractions, and Decreased Risk of Myocardial Infarction. New England Journal of Medicine 1993;329:1829-34. doi: 10.1056/NEJM199312163292501.
26. Mukamal KJ, Jensen MK, Grønbaek M, Stampfer MJ, Manson JE, Pischon T, Rimm EB. Drinking frequency, mediating biomarkers, and risk of myocardial infarction in women and men. Circulation 2005;112:1406-13. doi: 10.1161/CIRCULATIONAHA.105.537704.
27. Gaziano JM, Hennekens CH, Godfried SL, Sesso HD, Glynn RJ, Breslow JL, Buring JE. Type of alcoholic beverage and risk of myocardial infarction. The American journal of cardiology 1999;83:52-7. doi: S0002-9149(98)00782-6 [pii].

28. Mänttäri M, Tenkanen L, Alikoski T, Manninen V. Alcohol and coronary heart disease: the roles of HDL-cholesterol and smoking. Journal of internal medicine 1997;241:157-63.
29. Magnus P, Bakke E, Hoff DA, Høiseth G, Graff-Iversen S, Knudsen GP, Myhre R, Normann PT, Næss Ø, Tambs K, et al. Controlling for high-density lipoprotein cholesterol does not affect the magnitude of the relationship between alcohol and coronary heart disease. Circulation 2011;124:2296-302. doi: 10.1161/CIRCULATIONAHA.111.036491.
30. McClelland RL, Bild DE, Burke GL, Mukamal KJ, Lima JA, Kronmal RA. Alcohol and coronary artery calcium prevalence, incidence, and progression: Results from the Multi-Ethnic Study of Atherosclerosis (MESA). American Journal of Clinical Nutrition 2008;88:1593-601. doi: 10.3945/ajcn.2008.26420.

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Shue Huang, Li Junjuan, Yuntao Wu, Sareh Ranjbar, Aijun Xing, Haiyan Zhao, Yanxiu Wang, Gregory C. Shearer, Alice H. Lichtenstein, Shouling Wu, Xiang Gao. Tea consumption and longitudinal change in HDL cholesterol concentration in Chinese adults. Journal of the American Heart Association. 2018; 7 (13). Pii: e008814.

Shue Huang, Li Junjuan, Gregory C. Shearer, Alice H. Lichtenstein, Xiaoming Zheng, Yuntao Wu, Cheng Jin, Shouling Wu, and Xiang Gao. Longitudinal study on alcohol consumption and HDL concentrations: a community-based study. American Journal of Clinical Nutrition. 2017; 105:1-8

Zhe Huang, **Shue Huang (co-first),** Hongliang Cong, Zheng Li, Junjuan Li, Kathleen L Keller, Gregory C. Shearer, Penny M Kris-Etherton, Shouling Wu, Xiang Gao. Smell and taste dysfunction are associated with higher total cholesterol concentrations in Chinese adults. Journal of Nutrition. 2017; 147 (8): 1546-1551.

Timothy D. O'Connell, Robert C. Block, **Shue Huang**, Gregory C. Shearer. ω3-Polyunsaturated fatty acids for heart failure: Effects of dose on efficacy and novel signaling through free fatty acid receptor 4. Journal of Molecular and Cellular Cardiology. 2017; 103:74-92.

Shue Huang, Yi-Hsuan Liu, and Xiang Gao. Relationship of Olfactory and Taste Dysfunction to Dyslipidemia and Hypertension. SCAN'S Pulse. 2018 Fall; 37 (4): 1-4 **(Cover story)**

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