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Complete List of Authors:	Okwuosa, Tochi; Wayne State University, Internal Medicine, Division of Cardiology Klein, Oana; University of California, San Francisco, Chan, Cheeling; Northwestern University, Schreiner, Pamela; University of Minnesota, Liu, Kiang; Northwestern University, Green, David; Northwestern University,
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Long-Term Change in Alcohol Consumption Status and Variations in Serum Fibrinogen Levels: The Coronary Artery Risk Development in Young Adults (CARDIA) Study

Okwuosa: Fibrinogen Changes with Alcohol Consumption Status

Authors: Tochi M. Okwuosa, DO, FACC[1]; Oana Klein, MD, MS[2]; Cheeling Chan, MS[3]; Pamela Schreiner, PhD[4]; Kiang Liu, PhD[5]; David Green, MD, PhD[6].

[1] Assistant Professor, Director Preventive Cardiology, Wayne State University School of Medicine, Department of Medicine, Division of Cardiology, Detroit Michigan

[2] Assistant Clinical Professor, Department of Medicine, University of California San Francisco, Department of Medicine, San Francisco, California

[3] Statistical Analyst/ Programmer, Northwestern University Feinberg School of Medicine, Departments of Preventive Medicine and Medicine, Chicago, Illinois

[4] Professor and Director of Graduate Studies, University of Minnesota School of Public Health, Division of Epidemiology & Community Health, Minneapolis, Minnesota

[5] Professor in Preventive Medicine and Medicine-General Internal Medicine and Geriatrics, Northwestern University Feinberg School of Medicine, Departments of Preventive Medicine and Medicine, Chicago, Illinois

[6] Professor Emeritus in Medicine-Hematology/Oncology, Northwestern University Feinberg School of Medicine, Department of Medicine, Division of Hematology/Oncology, Chicago, Illinois

Correspondence:

Tochukwu E. M. Okwuosa, D.O., FACC

Assistant Professor of Medicine and Cardiology

Wayne State University – Harper University Hospital

3990 John R – 4 Hudson, Detroit, MI 48201

Telephone: 313.745.2620; Fax: 313.745.8643

Email: tokwuosa@med.wayne.edu

ABSTRACT

Objective: To examine long-term associations between change in alcohol consumption status and cessation of alcohol use, and blood fibrinogen levels in a large, young, biracial cohort.

Design: ANCOVA models were used to analyze participants within the CARDIA cohort who had fibrinogen and alcohol use data at year 7 (1992-93; ages 25-37) and year 20 examinations.

Setting: Four urban U.S. cities.

Patients: 2548 men and women within the CARDIA cohort.

Interventions: None

Main Outcome Measures: 13-year changes in alcohol use related to changes in fibrinogen.

Results: Over 13 years, mean fibrinogen increased by 71mg/dL vs. 70mg/dL (p=NS) in black men (BM) vs. white men (WM), and 78mg/dL vs. 68mg/dL ($p<0.05$) in black women (BW) vs. white women (WW), respectively. Compared with never-drinkers, there were smaller longitudinal increases in fibrinogen for BM, BW and WW (but larger increase in WM) who became or stayed drinkers, after multivariable adjustment. For BM, WM and WW, fibrinogen increased the most among persons who quit drinking over 13 years [$p<0.001$ for WM (fibrinogen increase = 86.5 (7.1) [mean (SE)]), compared with never-drinkers (fibrinogen increase = 53.1 (5.4)].

Conclusions: In this young cohort, compared to the participants who never drank, those who became/stayed drinkers had smaller increases, while those who quit drinking had the highest increase in fibrinogen over 13 years of follow-up. The results provide a novel insight into the mechanism for established protective effect of moderate alcohol intake on CVD outcomes.

Key Words: Fibrinogen, Alcohol, Cardiovascular Diseases, Risk factors, Young adults

INTRODUCTION

Numerous studies have linked moderate alcohol consumption with lower cardiovascular disease (CVD) morbidity and mortality.(1-3) Conversely, fibrinogen – the precursor of fibrin, a cofactor for platelet aggregation, and a major determinant of blood viscosity and atherogenesis – directly and independently correlates with CVD, as well as CVD risk factors.(4, 5) Many cross-sectional and prospective studies have found lower serum fibrinogen levels among alcohol consumers. (4, 6) Accordingly, fibrinogen levels decline with lifestyle interventions such as smoking cessation, exercise and moderate alcohol consumption.(4, 5, 7)

Alcohol is suggested to be causally related to lower risk of CVD through changes in lipids and hemostatic/inflammatory factors, such as fibrinogen.(1, 3, 6, 8, 9) This observed relationship between alcohol consumption and CVD follows a non-linear J-shaped curve, thus suggesting hazards to excessive alcohol consumption, and to complete abstinence.(1, 6, 10) In addition, there is some data in the literature linking moderate increase in alcohol consumption status to decreased risk of CVD and diabetes;(11, 12) and better health among moderate drinkers compared with alcohol quitters after acute myocardial infarction (MI).(13) Fibrinogen, lipids and other inflammatory and hemostatic factors implicated in CVD also appear to follow a J-shaped distribution in their relationship with alcohol consumption.(6, 9, 14) Nonetheless, there are very sparse data describing the relationship between long-term change in alcohol consumption status and any of these factors.(10) Even less is known about the influence of cessation of alcohol use on these factors.

We report 13-year longitudinal associations between change in alcohol consumption status and cessation of alcohol use, and variations in fibrinogen levels among a biracial group of young adult participants from the large CARDIA (Coronary Artery Risk Development in Young Adults Study) cohort. Findings from this study of young adults might provide some insight into the established protective effect of moderate alcohol intake on CVD outcomes. We stratified our findings by sex and race.

METHODS

Study participants: CARDIA is an ongoing multicenter prospective cohort study designed to investigate the evolution of CVD risk factors and subclinical atherosclerosis in young adults. Inclusion/exclusion criteria, baseline characteristics, and details of the study design, have been described elsewhere.(15) Briefly, in 1985-1986 the cohort enrolled 5115 black and white adults aged 18-30 years recruited from four urban U.S. areas (Birmingham, Alabama; Oakland, California; Chicago, Illinois and Minneapolis, Minnesota). Participants were balanced by age, sex, race, and education at baseline. Persons with coronary heart disease [CHD] (n=6), persons with non-fasting glucose and missing data for triglycerides and low density lipoprotein (LDL) cholesterol (n=312), and persons missing other covariates of interest (n=105) were excluded. The final cohort for analysis included 2548 participants. The institutional review boards at all the study sites approved the study protocol, and written informed consent was obtained from all study participants.

Fibrinogen was measured at year 7 ([Y7] our study baseline), and again at year 20 ([Y20] follow-up in this study). Of the 3844 participants examined at $baseline_{07}$, 804 persons were lost to follow-up. We included 2520 non-pregnant women and men with fibrinogen measurements, alcohol data and other covariates of interest at both baseline and follow-up.

Covariates ascertainment: Blood pressure, cholesterol, height, weight, waist circumference, smoking, and physical activity were measured in each examination using a standardized protocol.[18] Interviewer-administered questionnaires were used to obtain information on age, race, socioeconomic measures, diabetes history, cigarette smoking status, family history and medication use.(15)

Alcohol consumption: Alcohol use was assessed via interviewer-administered questionnaire for different types of alcoholic beverages (wine, beer, and liquor). Current alcohol drinkers were defined as

individuals who drank any alcoholic beverages in the past year. Otherwise, individuals were classified as non-drinkers.

Change in alcohol consumption status over 13 years was categorized according to dichotomized alcohol consumption groupings (non-drinker, current drinker) at Y7 and Y20 and four mutually exclusive groups to reflect long-term changes in alcohol consumption status were defined: continued non-drinker (individuals persistently in the "non-drinker" category at both Y7 and Y20, referent), became drinker (individuals in the "non-drinker" category at Y7 but in the "current" category at Y20), stayed drinker (individuals persistently in the "current" category at both Y7 and Y20), and quit drinking (individuals in the "current" category at Y7 but in the "non-drinker" category in Y20).

We used categorized change in alcohol use rather than a numeric value of change in alcohol consumption over time for 2 reasons: first, per the U.S. Department of Health and Human Services, alcohol use was categorized as none, moderate, and at-risk based on established thresholds; second, the distribution of changes in alcohol use (as numeric values) over time in the general population is skewed and not normally distributed. As such, changes in alcohol use cannot be analyzed using parametric statistical tests.

Fibrinogen measurement: Each participant had blood samples drawn after an 8-hour fast, between 7 a.m. and 10 a.m. Within 10 minutes of collection, repeated inversion was used to mix the samples, which were then spun in a refrigerated centrifuge at 4°C for 20 minutes. Within 90 minutes, the samples were stored at -70°C for a maximum of 4 months**.** In 2003, automated nephelometry was used to assay samples stored since Y7 (1992-93), as previously described.(16) This method was also used at Y20.

Statistical analysis: All analyses were performed by sex/race strata, with 2-sided $p<0.05$ considered statistically significant. Baseline and follow-up alcohol consumption status and pairwise differences in covariates by sex/race groups were estimated using t-tests, chi-square tests, and Fisher's exact tests, as

appropriate. ANCOVA models were used to relate changes in status of alcohol use (predictor variable) to changes in mean fibrinogen levels (outcome variable) over 13 years, with adjustments for covariates. Model 1 adjusted for baseline (year 7) age and fibrinogen level. Model 2 adjusted for baseline age, fibrinogen level, family history of heart disease, education, physical activity and traditional CVD risk factors (including hypertension, diabetes, dyslipidemia, hypertriglyceridemia, obesity and number of cigarettes/day); 13-year change in physical activity score, as well as follow-up statuses of traditional CVD risk factors as listed. We stratified our findings by sex/race groups because the CARDIA study group was designed to be balanced by age, sex, race and education when recruited at baseline. Analyses were conducted with SAS statistical software version 9.2 (SAS Institute Inc, Cary, NC).

RESULTS

Baseline Characteristics: Baseline characteristics for this sample have previously been described. In brief, we included 2548 participants (55% women, 43% black), mean age of 32.2 years (range 25-37 years) at study baseline. Supplemental Table 1 shows summary statistics for key variables at baseline and follow-up. Over 13 years, mean fibrinogen increased by 71mg/dL vs. 70mg/dL (p=NS) in black men compared with white men, and 78mg/dL vs. 68mg/dL ($p<0.05$) in black women compared with white women, respectively.

The prevalence of alcohol use at baseline and year 20 was higher among white men and women relative to black men and women (both $p<0.01$). Compared to study baseline, the prevalence of alcohol use at year 20 was higher among white men and women, but lower among black men and women by follow-up 13 years later (Supplemental Table 1).

Multivariable Changes in Fibrinogen Levels in Relation to Changes in Alcohol Consumption Status: The alcohol drinking status for most participants remained stable through the years (Table 1). More individuals remained non-drinker (N = 829) or stayed drinker (N = 1088), while fewer persons changed their drinking status through the years (N = 309 for those who became drinker, and 294 for those who quit drinking). After adjustments for various covariates (models 1 and 2 in Figure), changes in alcohol consumption status from study baseline to follow-up were inversely associated with changes in mean fibrinogen levels during the same time period. As such, becoming or staying a drinker (for both models) was associated with smaller mean increase in 13-year follow-up fibrinogen levels compared with never-drinkers. This held true among black men and women, but was particularly strongest in white women (all $p<0.001$ for both models among white women). An exception was white men, whose fibrinogen

increased more in those who became or stayed drinkers and increased the least among those who never drank alcohol over the 13 years.

For all alcohol use patterns studied, quitting alcohol use was associated with the *largest* mean increase in fibrinogen by the 13-year follow-up ($p<0.001$ for white men, compared with never-drinkers). For black women, change in fibrinogen was essentially the same for those who quit drinking relative to those who never drank alcohol over the years.

Our findings remained the same when at-risk drinkers – defined as ≥3 drinks on the day of maximum intake in the past month or ≥8 drinks per week for women; and ≥4 drinks on the day they drank the most in the past month or ≥15 drinks per week for men(17) – were excluded from the analysis (data not shown). Our findings also did not change when follow-up CVD risk factors were excluded from Model 2.

DISCUSSION

For the first time, we directly examined associations between changes in long-term alcohol consumption status and alcohol cessation, and changes in serum fibrinogen levels in a large, young population of black and white men and women. Overall we observed that fibrinogen rose less in persons who became drinkers or remained drinkers, and interestingly, increased more in persons who quit drinking. This pattern held for three of the sex/race groups in our study, even after adjusting for study baseline age, fibrinogen levels, family history of heart disease, education, physical activity and traditional CVD risk factors; 13-year changes in physical activity, as well as follow-up statuses of traditional CVD risk factors (as detailed in model 2 in the Figure). However, for white men, continued non-drinker status was not associated with a greater rise in fibrinogen.

Fibrinogen has shown significant independent positive associations with CHD, CVD and their risk factors – including age, smoking history, physical activity, body mass index, total and LDL cholesterol and systolic blood pressure,(4) (5) while the opposite is true for alcohol consumption. In fact, alcohol consumed in moderate quantities is inversely correlated with risk factors and mortality for CHD and CVD (including stroke).(2, 3)

We found that overall, persons who continued to use and those who initiated alcohol consumption during the 13 years of follow-up had smaller changes in fibrinogen levels relative to those who never consumed alcohol. Several prospective and cross-sectional studies have shown significant inverse associations between alcohol consumption and serum fibrinogen levels.(4, 6) Indeed, moderate alcohol consumption has been associated with platelet inhibition similar to that observed with aspirin use.(18) However, while some studies have examined the relationship between *very* short-term alcohol intake and changes in fibrinogen levels in a limited number of patients,(8, 19) very sparse data exist that have examined associations between long-term alcohol intake status and variations in fibrinogen levels

in a large population of participants. In addition, the youth of our study population contributes significant information to existing literature because it evaluates alcohol effects on CVD risk, with minimal confounding. Our study suggests that alcohol can still modulate CVD risk even in young adults, an important finding which agrees with benefits to CVD risk modulation beginning early in life.

An interesting and important observation from our study is that in all sex/race groups, among those who quit drinking over the years, fibrinogen levels increased to values higher than observed for never-drinkers and the rest of the drinking groups. Indeed, several studies have observed a J-shaped curve in the relationship between alcohol and CVD such that lower alcohol consumption was associated with reduced CVD, while the reverse was true for higher quantities of alcohol consumed.(1, 2, 20) A study of patients immediately post MI showed better health among patients with moderately increased alcohol intake relative to quitters.(13) Nonetheless, no study to our knowledge has investigated the effects of quitting alcohol consumption on inflammatory/thrombotic markers or CVD risk factors in general. Fibrinogen is a marker of platelet aggregation and vascular thrombosis. In acute, short-term human and experimental models, discontinuation of alcohol use has been associated with rebound platelet aggregation.(14, 18, 21)

Unlike the rest of the sex/race groups, white men who became drinkers and those who stayed drinkers through the years had larger increases in fibrinogen levels relative to never-drinkers. Prospective and cross-sectional studies have shown the observed J-curve pattern to beneficial effects of alcohol in whites, but not in blacks; and moderate alcohol drinking to be associated with reduced CVD mortality in whites, but not blacks.(22, 23) This finding in our study in unexplained, and requires further exploration.

The strength of our study lies in the longitudinal nature of our assessment with a relatively long follow-up period of 13 years, the large size of the study, the youth of the study participants (with few residual confounding factors at baseline), and the sex/racial makeup of the cohort. Our study findings

are particularly novel in that in examining the associations between alcohol consumption and fibrinogen changes in a large population of black and white men and women, we consolidated many years of alcohol use into each of the alcohol status categories; while examining their effects on variations in fibrinogen levels within each group. Thus, our study provides an important addition to the literature with respect to the long-term relationship between alcohol use and fibrinogen as a marker of CVD.

Limitations include the relatively wide range of alcohol intake levels included among persons classified as drinkers. Nonetheless, our findings remained the same after dropping at-risk levels. We caution that this analysis does not specifically measure all other potential conditions associated with elevated fibrinogen levels, although on average the CARDIA cohort was recruited to be a relatively healthy sample at baseline. Furthermore, some participants were excluded from our analysis due to missing data or loss to follow-up. Findings were unchanged when imputation analysis was performed in a prior study by our group which examined associations between fibrinogen and CV risk factors in the same population.

Conclusion: In this young cohort of black and white men and women with minimal baseline confounding factors, increase in fibrinogen was overall smaller among drinkers and larger among those who quit drinking, compared with those who remained alcohol-free for 13 years. These results need to be confirmed in other populations. Our study provides some valuable insight into the mechanism of established protective effect of moderate alcohol intake on CVD outcomes, and concurs with benefits to CVD risk modulation beginning early in life. Translation of our findings to associations with CHD/CVD events would be of great interest.

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Conflicts of Interest: None

Data Sharing Statement: No additional data

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Figure Legend: Adjusted Mean Increase in Fibrinogen in Relation to Changes in Alcohol Use over 13 Years by Sex- Race: the CARDIA Study, 1992-2006

Table 1: Adjusted Mean Changes in Fibrinogen in relation to Changes in Alcohol Consumption Status over 13 Years by Sex- Race: the CARDIA Study, 1992-2006[¶]

Change in risk factors	Change in fibrinogen (mg/dL)											
	Men						Women					
	Blacks, Mean Δ (SE)			Whites, Mean Δ (SE)			Blacks, Mean Δ (SE)			Whites, Mean Δ (SE)		
	N	Model 1	Model 2	N	Model 1	Model 2	N	Model 1	Model 2[§]	N	Model 1	Model 2[§]
Alcohol use												
Continued non-drinker (ref)	124	71.9 (6.0)	69.9 (6.1)	139	57.8 (5.4)	56.2 (5.5)	338	84.0 (4.4)	84.0 (4.6)	228	82.2 (4.9)	82.7 (4.9)
Became drinker	39	66.3 (10.8)	68.4 (10.7)	84	61.5 (7.0)	61.5 (7.0)	78	62.1 (9.2)[c]	62.8 (9.2)[c]	108	54.3 (7.0)[a]	53.6 (6.9)[a]
Stayed drinker	191	67.7 (4.9)	68.4 (4.9)	415	72.3 (3.1)[c]*	73.3 (3.2)[b]*	149	69.9 (6.7)	69.9 (6.9)	333	55.4 (4.0)[a]	56.2 (4.1)[a]
Quit drinking	65	89.9 (8.3)	90.6 (8.2)[c]	78	90.1 (7.2)[a]	87.5 (7.3)[a]	86	93.0 (8.8)	82.3 (8.8)	65	103.4 (9.0)[c]	98.4 (9.1)

[¶]Each risk factor represents a separate ANCOVA model. Ref=referent. SE=standard error.

Model 1: adjusted for baseline (age, and fibrinogen level). Model 2: all variables in model 1 and additionally adjusted for family history of heart disease, education, baseline (physical activity, number of cigarettes/d, and all other risk factors shown in table simultaneously). Changes in fibrinogen or risk factors were defined by changes over 13 years from baseline to year 20 (year 20 – year 7).

[§] Similar results were observed with addition of birth control pill or hormone use in the models.

*P<0.05, †P<0.01, ‡P<0.001 compared to Blacks between same model within gender.

[a]P <0.001, [b]P<0.01, [c]P<0.05 compared with the referent category of risk factor within sex/race.

Supplemental Table 1. Baselineand Year 20 (Follow-up) Characteristics by Sex- Race: the CARDIA Study, 1992-2006**

Variables	Men		Women	
	Black (N=428)	Whites (N=726)	Black (N=656)	Whites (N=738)
Baseline (Year 7)				
Age, y	31.6 (3.7)	32.7 (3.3)‡	31.6 (3.8)	32.7 (3.3)‡
Highest education attained, y	14.2 (2.3)	16.1 (2.6)‡	14.6 (2.1)	16.2 (2.4)‡
Family history of CVD, %	39.7	32.0†	43.3	33.3‡
Diastolic BP, mmHg	72.3 (9.8)	70.3 (9.0)‡	69.9 (10.3)	64.8 (8.0)‡
LDL cholesterol, mg/dL	111.8 (35.6)	113.7 (32.4)	104.5 (29.3)	101.9 (27.6)
HDL cholesterol, mg/dL	50.8 (14.2)	45.7 (10.9)‡	55.1 (13.7)	56.5 (12.5)*
Glucose, *u*g/dL	94.1 (22.7)	93.3 (10.8)	89.1 (15.3)	88.4 (10.2)
Triglycerides, mg/dL	81.6 (51.4)	97.2 (59.3)‡	67.0 (37.8)	69.7 (39.1)
Physical activity, exercise unit	477.6 (345.1)	414.2 (263.5)‡	231.5 (215.7)	309.5 (229.2)‡
BMI, kg/m^2	27.1 (5.2)	25.8 (4.0)‡	28.7 (7.3)	24.4 (5.1)‡
Fibrinogen, mg/dL	328.3 (72.7)	305.1 (56.8)‡	371.0 (80.9)	324.5 (67.0)‡
Current alcohol use, %	61.4	68.9†	36.3	54.2‡
Antihypertensive medication, %	2.1	1.1	2.9	0.4‡

Variables	Men		Women	
	Black (N=428)	Whites (N=726)	Black (N=656)	Whites (N=738)
Lipid-lowering medication, %	0.2	0.0	0.3	0.5
Current smoking, %	31.5	18.2‡	25.6	15.7‡
Obesity, %	24.1	12.8‡	36.9	12.7‡
Hypertension, %	6.1	4.1	6.7	0.8‡
Diabetes, %	1.6	0.7	1.1	0.5
Hypertriglyceridemia, %	4.0	6.5	0.9	1.4
Dyslipidemia, %	39.9	46.6*	46.8	39.6†
Year 20				
Current alcohol use, %	54.9	69.7‡	34.9	60.1‡
Antihypertensive medication, %	19.2	11.7‡	28.5	6.4‡
Lipid-lowering medication, %	7.9	13.4†	5.5	4.2
Current smoking, %	27.1	13.9‡	20.0	11.1‡
Obesity, %	43.9	26.3‡	57.8	24.9‡
Hypertension, %	25.0	14.9‡	34.9	8.3‡
Diabetes, %	10.5	4.4‡	10.1	2.6‡
Hypertriglyceridemia, %	6.8	16.8‡	2.9	5.0*
Dyslipidemia, %	52.8	58.5	48.3	37.3‡

Variables	Men		Women	
	Black (N=428)	Whites (N=726)	Black (N=656)	Whites (N=738)
13-y Difference (year 20 – year 7)				
Diastolic BP, mmHg	3.0 (11.7)	0.8 (9.4)‡	5.6 (12.3)	2.4 (9.1)‡
LDL cholesterol, mg/dL	0.1 (33.1)	1.1 (32.2)	3.3 (25.9)	4.3 (24.7)
HDL cholesterol, mg/dL	-1.1 (11.3)	1.0 (9.8)‡	2.1 (12.0)	5.1 (12.3)‡
Glucose, *u*g/dL	9.6 (29.5)	7.6 (16.9)	11.3 (26.6)	5.1 (13.7)‡
Triglycerides, mg/dL	24.1 (57.4)	29.9 (63.3)	21.8 (42.7)	24.9 (48.0)
Physical activity, exercise unit	-51.5 (345.2)	-1.3 (260.1)†	-9.9 (248.4)	27.1 (235.9)†
BMI, kg/m^2	2.9 (3.6)	2.5 (4.9)	3.8 (4.5)	2.7 (4.1)‡
Fibrinogen, mg/dL	71.0 (71.2)	69.5 (67.8)	78.1 (86.2)	67.8 (77.1)*

*P<0.05, †P<0.01, ‡P<0.001 compared with blacks between the value of the characteristic within gender.

§ Data are given as means (SD) unless otherwise specified.

‖ Abbreviations: CVD, cardiovascular disease (included heart attack and stroke); BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; BMI, body mass index

Definitions: Current smoking - at least 5 cigarettes per week almost every week for at least 3 months, Diabetes - fasting glucose ≥126 mg/dL or taking diabetic medication; hypertension - systolic blood pressure ≥140mmHg, diastolic blood pressure (DBP) ≥90mmHg, or taking anti-hypertensive medication; dyslipidemia - low HDL cholesterol (<40mg/dL [men] or <50mg/dL [women]) and/or high LDL cholesterol (>130mg/dL) or taking lipid-lowering medication, hypertriglyceridemia - triglycerides >200mg/dL; obesity was defined as BMI ≥30kg/m^2.

** Baseline refers to year 7 of the CARDIA cohort

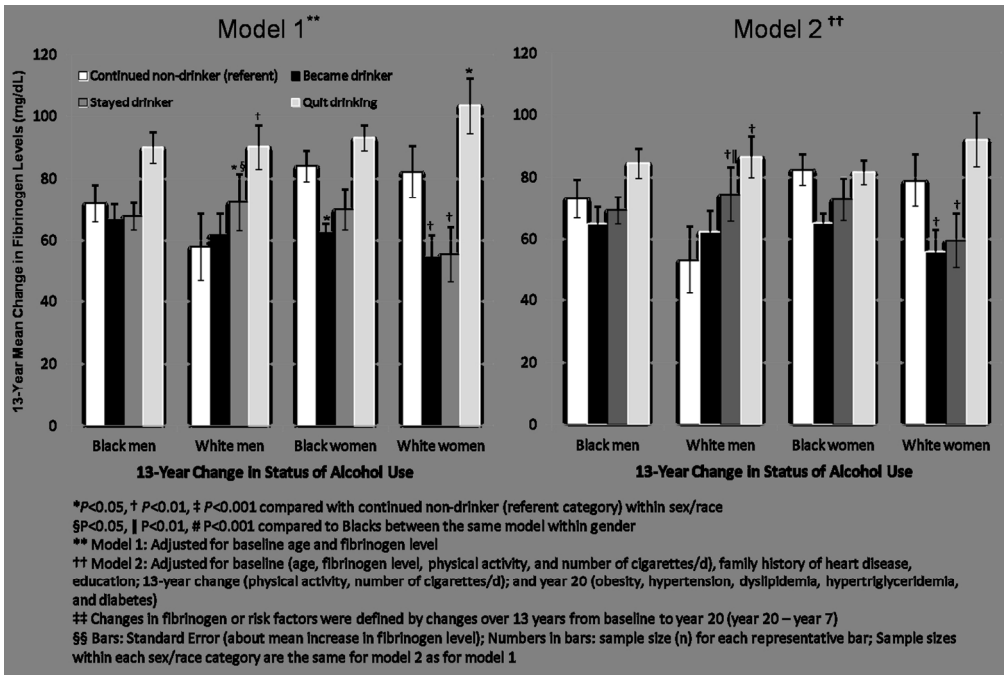

Figure Legend: Adjusted Mean Increase in Fibrinogen in Relation to Changes in Alcohol Use over 13 Years by Sex- Race: the CARDIA Study, 1992-2006
249x166mm (150 x 150 DPI)

Long-Term Change in Alcohol Consumption Status and Variations in Serum Fibrinogen Levels: The Coronary Artery Risk Development in Young Adults (CARDIA) Study

Okwuosa: Fibrinogen Changes with Alcohol Consumption Status

Article Summary

Article Focus:

- To gain some insight into the established protective effect of moderate alcohol intake on CVD outcomes.
- To determine the 13-year longitudinal associations between change in alcohol consumption status and cessation of alcohol use.
- To determine variations in fibrinogen levels among a biracial group of young adult participants from the large CARDIA (Coronary Artery Risk Development in Young Adults Study) cohort.

Key Messages:

- In this young cohort of black and white men and women with minimal baseline confounding factors, compared to the participants who never drank, those who became/stayed drinkers had smaller increases; while those who quit drinking had the highest increase in fibrinogen over 13 years of follow-up.

Strengths/Limitations:

The strength of our study lies in the longitudinal nature of our assessment with a relatively long follow-up period of 13 years, the large size of the study, the youth of the study participants (with few residual confounding factors at baseline), and the sex/racial makeup of the cohort. Our study findings are particularly novel in that in examining the associations between alcohol consumption and fibrinogen changes in a large population of black and white men and women, we consolidated many years of

alcohol use into each of the alcohol status categories; while examining their effects on variations in fibrinogen levels within each group. Thus, our study provides an important addition to the literature with respect to the long-term relationship between alcohol use and fibrinogen as a marker of CVD.

Limitations include the relatively wide range of alcohol intake levels included among persons classified as drinkers. Nonetheless, our findings remained the same after dropping at-risk levels. We caution that this analysis does not specifically measure all other potential conditions associated with elevated fibrinogen levels, although on average the CARDIA cohort was recruited to be a relatively healthy sample at baseline. Furthermore, some participants were excluded from our analysis due to missing data or loss to follow-up. Findings were unchanged when imputation analysis was performed in a prior study by our group which examined associations between fibrinogen and CV risk factors in the same population.

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, explain how loss to follow-up was addressed
		(*e*) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) Summarise follow-up time (eg, average and total amount)
Outcome data	15*	Report numbers of outcome events or summary measures over time
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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Long-Term Change in Alcohol Consumption Status and Variations in Fibrinogen Levels: The Coronary Artery Risk Development in Young Adults (CARDIA) Study

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Long-Term Change in Alcohol Consumption Status and Variations in Fibrinogen Levels: The Coronary Artery Risk Development in Young Adults (CARDIA) Study

Okwuosa: Fibrinogen Changes with Alcohol Consumption Status

Authors: Tochi M. Okwuosa, DO, FACC[1]; Oana Klein, MD, MS[2]; Cheeling Chan, MS[3]; Pamela Schreiner, PhD[4]; Kiang Liu, PhD[5]; David Green, MD, PhD[6].

[1] Assistant Professor, Director Preventive Cardiology, Wayne State University School of Medicine, Department of Medicine, Division of Cardiology, Detroit Michigan

[2] Assistant Clinical Professor, Department of Medicine, University of California San Francisco, Department of Medicine, San Francisco, California

[3] Statistical Analyst/ Programmer, Northwestern University Feinberg School of Medicine, Departments of Preventive Medicine and Medicine, Chicago, Illinois

[4] Professor and Director of Graduate Studies, University of Minnesota School of Public Health, Division of Epidemiology & Community Health, Minneapolis, Minnesota

[5] Professor in Preventive Medicine and Medicine-General Internal Medicine and Geriatrics, Northwestern University Feinberg School of Medicine, Departments of Preventive Medicine and Medicine, Chicago, Illinois

[6] Professor Emeritus in Medicine-Hematology/Oncology, Northwestern University Feinberg School of Medicine, Department of Medicine, Division of Hematology/Oncology, Chicago, Illinois

Correspondence:

Tochukwu E. M. Okwuosa, D.O., FACC

Assistant Professor of Medicine and Cardiology

Wayne State University – Harper University Hospital

3990 John R – 4 Hudson, Detroit, MI 48201

Telephone: 313.745.2620; Fax: 313.745.8643

Email: tokwuosa@med.wayne.edu

Article Summary

Article Focus:

- To gain some insight into the established protective effect of moderate alcohol intake on CVD outcomes.
- To determine the 13-year longitudinal associations between change in alcohol consumption status and cessation of alcohol use.
- To determine variations in fibrinogen levels among a biracial group of young adult participants from the large CARDIA (Coronary Artery Risk Development in Young Adults Study) cohort.

Key Messages:

- In this young cohort of black and white men and women with minimal baseline confounding factors, compared to the participants who never drank, those who became/stayed drinkers had smaller increases; while those who quit drinking had the highest increase in fibrinogen over 13 years of follow-up.

Strengths/Limitations:

The strength of our study lies in the longitudinal nature of our assessment with a relatively long follow-up period of 13 years, the large size of the study, the youth of the study participants (with few residual confounding factors at baseline), and the sex/racial makeup of the cohort. Our study findings are particularly novel in that in examining the associations between alcohol consumption and fibrinogen changes in a large population of black and white men and women, we consolidated many years of alcohol use into each of the alcohol status categories; while examining their effects on variations in fibrinogen levels within each group. Thus, our study provides an important addition to the literature with respect to the long-term relationship between alcohol use and fibrinogen as a marker of CVD.

Limitations include the relatively wide range of alcohol intake levels included among persons classified as drinkers. Nonetheless, our findings remained the same after dropping at-risk levels. We

caution that this analysis does not specifically measure all other potential conditions associated with elevated fibrinogen levels, although on average the CARDIA cohort was recruited to be a relatively healthy sample at baseline. Furthermore, some participants were excluded from our analysis due to missing data or loss to follow-up. Findings were unchanged when imputation analysis was performed in a prior study by our group which examined associations between fibrinogen and CV risk factors in the same population.

ABSTRACT

Objective: To examine long-term associations between change in alcohol consumption status and cessation of alcohol use, and fibrinogen levels in a large, young, biracial cohort.

Design: ANCOVA models were used to analyze participants within the CARDIA cohort who had fibrinogen and alcohol use data at year 7 (1992-93; ages 25-37) and year 20 examinations.

Setting: Four urban U.S. cities.

Patients: 2520 men and women within the CARDIA cohort.

Main Outcome Measures: 13-year changes in alcohol use related to changes in fibrinogen.

Results: Over 13 years, mean fibrinogen increased by 71mg/dL vs. 70mg/dL (p=NS) in black men (BM) vs. white men (WM), and 78mg/dL vs. 68mg/dL ($p<0.05$) in black women (BW) vs. white women (WW), respectively. Compared with never-drinkers, there were smaller longitudinal increases in fibrinogen for BM, BW and WW (but larger increase in WM) who became or stayed drinkers, after multivariable adjustment. For BM, WM and WW, fibrinogen increased the most among persons who quit drinking

over 13 years [p<0.001 for WM (fibrinogen increase = 86.5 (7.1) [mean (SE)]), compared with never-drinkers (fibrinogen increase = 53.1 (5.4)].

Conclusions: In this young cohort, compared to the participants who never drank, those who became/stayed drinkers had smaller increases, while those who quit drinking had the highest increase in fibrinogen over 13 years of follow-up. The results provide a novel insight into the mechanism for established protective effect of moderate alcohol intake on CVD outcomes.

Key Words: Fibrinogen, Alcohol, Cardiovascular Diseases, Risk factors, Young adults

INTRODUCTION

Numerous studies have linked moderate alcohol consumption with lower cardiovascular disease (CVD) morbidity and mortality.(1-3) Conversely, fibrinogen – the precursor of fibrin, a cofactor for platelet aggregation, and a major determinant of blood viscosity and atherogenesis – directly and independently correlates with CVD, as well as CVD risk factors.(4, 5) Many cross-sectional and prospective studies have found lower fibrinogen levels among alcohol consumers. (4, 6) Accordingly, fibrinogen levels decline with lifestyle interventions such as smoking cessation, exercise and moderate alcohol consumption.(4, 5, 7)

Alcohol is suggested to be causally related to lower risk of CVD through changes in lipids and hemostatic/inflammatory factors, such as fibrinogen.(1, 3, 6, 8, 9) This observed relationship between alcohol consumption and CVD follows a non-linear J-shaped curve, thus suggesting hazards to excessive alcohol consumption, and to complete abstinence.(1, 6, 10) In addition, there is some data in the literature linking moderate increase in alcohol consumption status to decreased risk of CVD and diabetes;(11, 12) and better health among moderate drinkers compared with alcohol quitters after acute myocardial infarction (MI).(13) Fibrinogen, lipids and other inflammatory and hemostatic factors implicated in CVD also appear to follow a J-shaped distribution in their relationship with alcohol consumption.(6, 9, 14) Nonetheless, there are very sparse data describing the relationship between long-term change in alcohol consumption status and any of these factors.(10) Even less is known about the influence of cessation of alcohol use on these factors.

We report 13-year longitudinal associations between change in alcohol consumption status and cessation of alcohol use, and variations in fibrinogen levels among a biracial group of young adult participants from the large CARDIA (Coronary Artery Risk Development in Young Adults Study) cohort. Findings from this study of young adults might provide some insight into the established protective effect of moderate alcohol intake on CVD outcomes. We stratified our findings by sex and race.

METHODS

Study participants: CARDIA is an ongoing multicenter prospective cohort study designed to investigate the evolution of CVD risk factors and subclinical atherosclerosis in young adults. Inclusion/exclusion criteria, baseline characteristics, and details of the study design, have been described elsewhere.(15) Briefly, in 1985-1986 the cohort enrolled 5115 black and white adults aged 18-30 years recruited from four urban U.S. areas (Birmingham, Alabama; Oakland, California; Chicago, Illinois and Minneapolis, Minnesota). Participants were balanced by age, sex, race, and education at baseline. The institutional review boards at all the study sites approved the study protocol, and written informed consent was obtained from all study participants.

Our study included 2971 non-pregnant CARDIA women and men with fibrinogen measurements at examination years 7 (Y7 – our study baseline) and 20 (Y20 – termed followup in our study). Persons with coronary heart disease [CHD] (n=6), persons with non-fasting glucose and missing data for triglycerides and low density lipoprotein (LDL) cholesterol (n=312), persons with missing changes in alcohol use status (n=28), and persons missing other covariates of interest (n=105) were excluded. The final cohort for analysis included 2520 non-pregnant women and men.

Covariates ascertainment: Blood pressure, cholesterol, height, weight, waist circumference, smoking, and physical activity were measured in each examination using a standardized protocol.[18] Interviewer-administered questionnaires were used to obtain information on age, race, socioeconomic measures, diabetes history, cigarette smoking status, family history and medication use.(15)

Alcohol consumption: Alcohol use was assessed via interviewer-administered questionnaire for different types of alcoholic beverages (wine, beer, and liquor). Current alcohol drinkers were defined as individuals who drank any alcoholic beverages in the past year. Otherwise, individuals were classified as non-drinkers.

Change in alcohol consumption status over 13 years was categorized according to dichotomized alcohol consumption groupings (non-drinker, current drinker) at Y7 and Y20 and four mutually exclusive groups to reflect long-term changes in alcohol consumption status were defined: continued non-drinker (individuals persistently in the "non-drinker" category at both Y7 and Y20, referent), became drinker (individuals in the "non-drinker" category at Y7 but in the "current" category at Y20), stayed drinker (individuals persistently in the "current" category at both Y7 and Y20), and quit drinking (individuals in the "current" category at Y7 but in the "non-drinker" category in Y20).

We used categorized change in alcohol use rather than a numeric value of change in alcohol consumption over time for 2 reasons: first, per the U.S. Department of Health and Human Services, alcohol use was categorized as none, moderate, and at-risk based on established thresholds; second, the distribution of changes in alcohol use (as numeric values) over time in the general population is skewed and not normally distributed. As such, changes in alcohol use cannot be analyzed using parametric statistical tests.

Fibrinogen measurement: Each participant had blood samples drawn after an 8-hour fast, between 7 a.m. and 10 a.m. Within 10 minutes of collection, repeated inversion was used to mix the samples, which were then spun in a refrigerated centrifuge at 4°C for 20 minutes. Within 90 minutes, the samples were stored at -70°C for a maximum of 4 months**.** Fibrinogen was measured in Y7 and Y20 plasma samples as previously described, using the BNII Nephelometer 100 Analyzer, Dade Behring, Deerfield, IL, USA (16). The assay was calibrated with a reference plasma of known fibrinogen concentration, and the intra-assay and inter-assay coefficient of variation were 2.7% and 2.6% at Y7 and 3.1% and 4.2% at Y20.

Statistical analysis: All analyses were performed by sex/race strata, with 2-sided $p<0.05$ considered statistically significant. Baseline and follow-up alcohol consumption status and pairwise differences in covariates by sex/race groups were estimated using t-tests, chi-square tests, and Fisher's exact tests, as

appropriate. ANCOVA models were used to relate changes in status of alcohol use (predictor variable) to changes in mean fibrinogen levels (outcome variable) over 13 years, with adjustments for covariates. Model 1 adjusted for baseline (Y7) age and fibrinogen level. Model 2 adjusted for baseline age, fibrinogen level, family history of heart disease, education, physical activity and traditional CVD risk factors (including hypertension, diabetes, dyslipidemia, hypertriglyceridemia, obesity and number of cigarettes/day); 13-year change in physical activity score, as well as follow-up statuses of traditional CVD risk factors as listed. We stratified our findings by sex/race groups because the CARDIA study group was designed to be balanced by age, sex, race and education when recruited at baseline. Analyses were conducted with SAS statistical software version 9.2 (SAS Institute Inc, Cary, NC).

RESULTS

Baseline Characteristics: Baseline characteristics for this sample have previously been described. In brief, we included 2520 participants (55% women, 43% black), mean age of 32.2 years (range 25-37 years) at study baseline. Supplemental Table 1 shows summary statistics for key variables at baseline and follow-up. Over 13 years, mean fibrinogen increased by 71mg/dL vs. 70mg/dL (p=NS) in black men compared with white men, and 78mg/dL vs. 68mg/dL ($p<0.05$) in black women compared with white women, respectively.

The prevalence of alcohol use at baseline and year 20 was higher among white men and women relative to black men and women (both $p<0.01$). Compared to study baseline, the prevalence of alcohol use at Y20 was higher among white men and women, but lower among black men and women by follow-up 13 years later (Supplemental Table 1).

Multivariable Changes in Fibrinogen Levels in Relation to Changes in Alcohol Consumption Status: The alcohol drinking status for most participants remained stable through the yearsFigure. More individuals remained non-drinker (N = 829) or stayed drinker (N = 1088), while fewer persons changed their drinking status through the years (N = 309 for those who became drinker, and 294 for those who quit drinking). After adjustments for various covariates (models 1 and 2 in Figure), changes in alcohol consumption status from study baseline to follow-up were inversely associated with changes in mean fibrinogen levels during the same time period. As such, becoming or staying a drinker (for both models) was associated with smaller mean increase in 13-year follow-up fibrinogen levels compared with never-drinkers. This held true among black men and women, but was particularly strongest in white women (all $p<0.001$ for both models among white women). An exception was white men, whose fibrinogen

increased more in those who became or stayed drinkers and increased the least among those who never drank alcohol over the 13 years.

For all alcohol use patterns studied, quitting alcohol use was associated with the *largest* mean increase in fibrinogen by the 13-year follow-up ($p<0.001$ for white men, compared with never-drinkers). For black women, change in fibrinogen was essentially the same for those who quit drinking relative to those who never drank alcohol over the years.

Our findings remained the same when at-risk drinkers – defined as ≥3 drinks on the day of maximum intake in the past month or ≥8 drinks per week for women; and ≥4 drinks on the day they drank the most in the past month or ≥15 drinks per week for men(17) – were excluded from the analysis (data not shown). Our findings also did not change when follow-up CVD risk factors were excluded from Model 2. Furthermore, the change in fibrinogen levels among participants who became or stayed drinker through the years remained significantly lower compared with the change among those who quit drinking – used as the referent group in this case (data not shown).

The health characteristics of the participants by alcohol consumption category are shown in Table 1. The unadjusted data show that at baseline (Y7), the continued non-drinker population and those who quit drinking had significantly higher prevalence of high blood pressure – which increased and remained significant by Y20 – compared with those who became or stayed drinker . Those who quit drinking had a significantly lower prevalence of diabetes at baseline, which increased (but not significantly) by followup at Y20. Interestingly, other assessed characteristics (including liver disease, hepatitis, digestive disease and cancer) were not significantly different among the alcohol consumption groups.

DISCUSSION

We directly examined associations between changes in long-term alcohol consumption and alcohol cessation, and changes in fibrinogen levels in a large, young population of black and white men and women. Overall we observed that fibrinogen rose less in persons who became drinkers or remained drinkers, and interestingly, increased more in persons who quit drinking. This pattern held for three of the sex/race groups in our study, even after adjusting for study baseline age, fibrinogen levels, family history of heart disease, education, physical activity and traditional CVD risk factors; 13-year changes in physical activity, as well as follow-up statuses of traditional CVD risk factors (as detailed in model 2 in the Figure). However, for white men, continued non-drinker status was not associated with a greater rise in fibrinogen.

Fibrinogen is mainly synthesized in the liver, and is a soluble glycoprotein which regulates plasma viscosity, induces reversible red cell aggregation and is the most abundant component of thrombi.(18, 19) In addition, fibrinogen increases platelet reactivity by binding glycoprotein IIb/IIIa receptor on the platelet surface.(18) Fibrin is an important component of atherogenesis and atheroma growth, and additionally provides a scaffold for smooth muscle cell proliferation and migration which attracts leukocytes, affecting endothelial permeability and vascular tone.(18, 20) Fibrinogen binds LDL cholesterol and lipids, and is consequently involved in the formation of the atherosclerotic lipid core.(18) Moderate alcohol consumption has beneficial effects on atherosclerosis, attributed to its anti-inflammatory and antioxidant effects, and to its actions on vascular function. These effects are thought to be mediated by polyphenols(21)

Fibrinogen has shown significant independent positive associations with CHD, CVD and their risk factors – including age, smoking history, physical activity, body mass index, total and LDL cholesterol and systolic blood pressure,(4) (5) while the opposite is true for alcohol consumption. In fact, alcohol consumed in moderate quantities is inversely correlated with risk factors and mortality for CHD and CVD (including stroke).(2, 3)

We found that overall, persons who continued to use and those who initiated alcohol consumption during the 13 years of follow-up had smaller changes in fibrinogen levels relative to those who never consumed alcohol. Several prospective and cross-sectional studies have shown significant inverse associations between alcohol consumption and fibrinogen levels.(4, 6) Indeed, moderate alcohol consumption has been associated with platelet inhibition similar to that observed with aspirin use.(22) However, while some studies have examined the relationship between *very* short-term alcohol intake and changes in fibrinogen levels in a limited number of patients,(8, 23) very sparse data exist that have examined associations between long-term alcohol intake status and variations in fibrinogen levels in a large population of participants. In addition, the youth of our study population contributes significant information to existing literature because it evaluates alcohol effects on CVD risk, with minimal confounding. Our study suggests that moderate alcohol consumption can still modulate CVD risk even in young adults, an important finding which agrees with benefits to CVD risk modulation beginning early in life. It also suggests fibrinogen as a possible mediator of this process.

An interesting and important observation from our study is that in all sex/race groups, among those who quit drinking over the years, fibrinogen levels increased to values higher than observed for never-drinkers and the rest of the drinking groups. Indeed, several studies have observed a J-shaped curve in the relationship between alcohol and CVD such that lower alcohol consumption was associated with reduced CVD, while the reverse was true for higher quantities of alcohol consumed.(1, 2, 24) A study of patients immediately post MI showed better health among patients with moderately increased alcohol intake relative to quitters.(13) Nonetheless, no study to our knowledge has investigated the effects of quitting alcohol consumption on inflammatory/thrombotic markers or CVD risk factors in general. Fibrinogen is a marker of platelet aggregation and vascular thrombosis. In acute, short-term human and experimental models, discontinuation of alcohol use has been associated with rebound platelet aggregation.(14, 22, 25)

Unlike the rest of the sex/race groups, white men who became drinkers and those who stayed drinkers through the years had larger increases in fibrinogen levels relative to never-drinkers. Prospective and cross-sectional studies have shown the observed J-curve pattern to beneficial effects of alcohol in whites, but not in blacks; and moderate alcohol drinking to be associated with reduced CVD mortality in whites, but not blacks.(26, 27) This finding in our study in unexplained, and requires further exploration.The strength of our study lies in the longitudinal nature of our assessment with a relatively long follow-up period of 13 years, the large size of the study, the youth of the study participants (with few residual confounding factors at baseline), and the sex/racial makeup of the cohort. Our study findings are particularly novel in that in examining the associations between alcohol consumption and fibrinogen changes in a large population of black and white men and women, we consolidated many years of alcohol use into each of the alcohol status categories; while examining their effects on variations in fibrinogen levels within each group. Thus, our study provides an important addition to the literature with respect to the long-term relationship between alcohol use and fibrinogen as a marker of CVD.

Limitations include the relatively wide range of alcohol intake levels included among persons classified as drinkers. Nonetheless, our findings remained the same after dropping at-risk levels. We caution that this analysis does not specifically measure all other potential conditions associated with elevated fibrinogen levels, although on average the CARDIA cohort was recruited to be a relatively healthy sample at baseline. Furthermore, some participants were excluded from our analysis due to missing data or loss to follow-up. Findings were unchanged when imputation analysis was performed in a prior study by our group which examined associations between fibrinogen and CV risk factors in the same population.

Conclusion: In this young cohort of black and white men and women with minimal baseline confounding factors, increase in fibrinogen was overall smaller among drinkers and larger among those who quit drinking, compared with those who remained alcohol-free for 13 years. These results need to be confirmed in other populations. Our study provides insight into the mechanism and possible role of fibrinogen on the established protective effect of moderate alcohol intake on CVD outcomes, and concurs with benefits to CVD risk modulation beginning early in life. Translation of our findings to associations with CHD/CVD events would be of great interest.

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Conflicts of Interest: None

Data Sharing Statement: No additional data

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Figure Legend: Adjusted Mean Increase in Fibrinogen in Relation to Changes in Alcohol Use over 13 Years by Sex- Race: the CARDIA Study, 1992-2006

Table 1. Year 7 and 20 Health Characteristics of Participants by Changes in Alcohol Consumption Status

Comorbidities*	Year 7					Year 20				
	Continued non-drinker	Became drinker	Stayed drinker	Quit drinking	P value†	Continued non-drinker	Became drinker	Stayed drinker	Quit drinking	P value†
High blood pressure (%)	11.0	5.8	7.7	10.5	0.012	27.1	15.2	19.3	28.9	<0.001
Stroke or TIA (%)	--	--	--	--	--	0.7	0.6	0.6	1.7	0.318
Diabetes (%)	4.7	3.2	1.7	2.0	0.001	9.2	5.8	5.6	9.5	0.007
Liver disease (%)	1.2	2.6	1.3	1.0	0.281	1.9	3.6	2.1	3.7	0.161
Hepatitis (%)	1.0	2.6	1.2	1.0	0.165	1.3	1.9	1.4	2.4	0.421
Digestive disease (%)	5.5	7.1	4.1	6.5	0.116	7.1	7.4	7.2	9.5	0.554
Cancer (%)	2.3	1.6	1.9	3.1	0.591	4.5	6.8	5.5	4.8	0.421
Mental disorder (%)	4.1	5.8	3.9	4.1	0.500	5.8	9.1	5.6	6.5	0.152

*Participants were asked if a physician had previously diagnosed them with the chronic conditions.

†Overall P values calculated using χ^2 test

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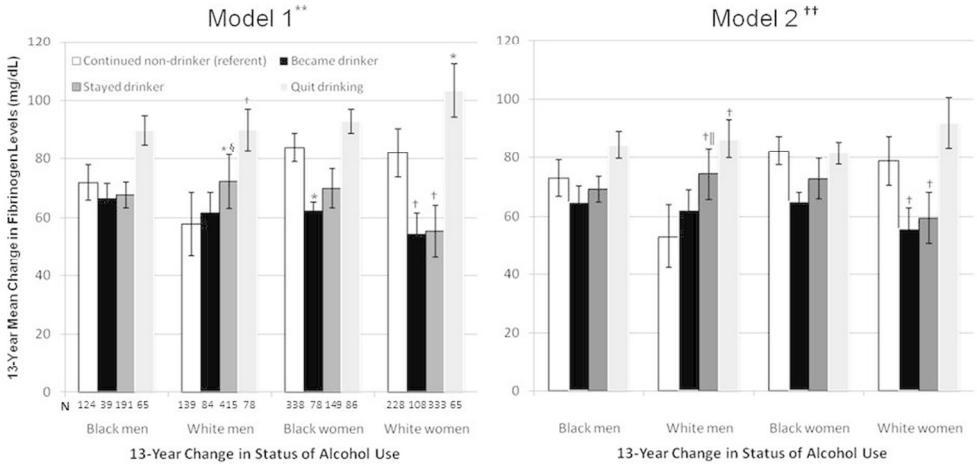

*P<0.05, † P<0.01, ‡ P<0.001 compared with continued non-drinker (referent category) within sex/race
§P<0.05, ‖ P<0.01, # P<0.001 compared to Blacks between the same model within gender
** Model 1: Adjusted for baseline age and fibrinogen level
†† Model 2: Adjusted for baseline (age, fibrinogen level, physical activity, and number of cigarettes/d), family history of heart disease, education; 13-year change (physical activity, number of cigarettes/d); and year 20 (obesity, hypertension, dyslipidemia, hypertriglyceridemia, and diabetes)
‡‡ Changes in fibrinogen or risk factors were defined by changes over 13 years from baseline to year 20 (year 20 – year 7)
§§ Bars: Standard Error (about mean increase in fibrinogen level); Numbers in bars: sample size (n) for each representative bar; Sample sizes within each sex/race category are the same for model 2 as for model 1

119x90mm (300 x 300 DPI)

Supplemental Table 1. Baselineand Year 20 (Follow-up) Characteristics by Sex- Race: the CARDIA Study, 1992-2006**

Variables	Men		Women	
	Black (N=428)	Whites (N=726)	Black (N=656)	Whites (N=738)
Baseline (Year 7)				
Age, y	31.6 (3.7)	32.7 (3.3)‡	31.6 (3.8)	32.7 (3.3)‡
Highest education attained, y	14.2 (2.3)	16.1 (2.6)‡	14.6 (2.1)	16.2 (2.4)‡
Family history of CVD, %	39.7	32.0†	43.3	33.3‡
Diastolic BP, mmHg	72.3 (9.8)	70.3 (9.0)‡	69.9 (10.3)	64.8 (8.0)‡
LDL cholesterol, mg/dL	111.8 (35.6)	113.7 (32.4)	104.5 (29.3)	101.9 (27.6)
HDL cholesterol, mg/dL	50.8 (14.2)	45.7 (10.9)‡	55.1 (13.7)	56.5 (12.5)*
Glucose, *u*g/dL	94.1 (22.7)	93.3 (10.8)	89.1 (15.3)	88.4 (10.2)
Triglycerides, mg/dL	81.6 (51.4)	97.2 (59.3)‡	67.0 (37.8)	69.7 (39.1)
Physical activity, exercise unit	477.6 (345.1)	414.2 (263.5)‡	231.5 (215.7)	309.5 (229.2)‡
BMI, kg/m^2	27.1 (5.2)	25.8 (4.0)‡	28.7 (7.3)	24.4 (5.1)‡
Fibrinogen, mg/dL	328.3 (72.7)	305.1 (56.8)‡	371.0 (80.9)	324.5 (67.0)‡
Current alcohol use, %	61.4	68.9†	36.3	54.2‡
Antihypertensive medication, %	2.1	1.1	2.9	0.4‡

Variables	Men		Women	
	Black (N=428)	Whites (N=726)	Black (N=656)	Whites (N=738)
Lipid-lowering medication, %	0.2	0.0	0.3	0.5
Current smoking, %	31.5	18.2‡	25.6	15.7‡
Obesity, %	24.1	12.8‡	36.9	12.7‡
Hypertension, %	6.1	4.1	6.7	0.8‡
Diabetes, %	1.6	0.7	1.1	0.5
Hypertriglyceridemia, %	4.0	6.5	0.9	1.4
Dyslipidemia, %	39.9	46.6*	46.8	39.6†
Year 20				
Current alcohol use, %	54.9	69.7‡	34.9	60.1‡
Antihypertensive medication, %	19.2	11.7‡	28.5	6.4‡
Lipid-lowering medication, %	7.9	13.4†	5.5	4.2
Current smoking, %	27.1	13.9‡	20.0	11.1‡
Obesity, %	43.9	26.3‡	57.8	24.9‡
Hypertension, %	25.0	14.9‡	34.9	8.3‡
Diabetes, %	10.5	4.4‡	10.1	2.6‡
Hypertriglyceridemia, %	6.8	16.8‡	2.9	5.0*
Dyslipidemia, %	52.8	58.5	48.3	37.3‡

Variables	Men		Women	
	Black (N=428)	Whites (N=726)	Black (N=656)	Whites (N=738)
13-y Difference (year 20 – year 7)				
Diastolic BP, mmHg	3.0 (11.7)	0.8 (9.4)‡	5.6 (12.3)	2.4 (9.1)‡
LDL cholesterol, mg/dL	0.1 (33.1)	1.1 (32.2)	3.3 (25.9)	4.3 (24.7)
HDL cholesterol, mg/dL	-1.1 (11.3)	1.0 (9.8)‡	2.1 (12.0)	5.1 (12.3)‡
Glucose, *u*g/dL	9.6 (29.5)	7.6 (16.9)	11.3 (26.6)	5.1 (13.7)‡
Triglycerides, mg/dL	24.1 (57.4)	29.9 (63.3)	21.8 (42.7)	24.9 (48.0)
Physical activity, exercise unit	-51.5 (345.2)	-1.3 (260.1)†	-9.9 (248.4)	27.1 (235.9)†
BMI, kg/m^2	2.9 (3.6)	2.5 (4.9)	3.8 (4.5)	2.7 (4.1)‡
Fibrinogen, mg/dL	71.0 (71.2)	69.5 (67.8)	78.1 (86.2)	67.8 (77.1)*

*P<0.05, †P<0.01, ‡P<0.001 compared with blacks between the value of the characteristic within gender.

§ Data are given as means (SD) unless otherwise specified.

‖ Abbreviations: CVD, cardiovascular disease (included heart attack and stroke); BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; BMI, body mass index

Definitions: Current smoking - at least 5 cigarettes per week almost every week for at least 3 months, Diabetes - fasting glucose ≥126 mg/dL or taking diabetic medication; hypertension - systolic blood pressure ≥140mmHg, diastolic blood pressure (DBP) ≥90mmHg, or taking anti-hypertensive medication; dyslipidemia - low HDL cholesterol (<40mg/dL [men] or <50mg/dL [women]) and/or high LDL cholesterol (>130mg/dL) or taking lipid-lowering medication, hypertriglyceridemia - triglycerides >200mg/dL; obesity was defined as BMI ≥30kg/m^2.

** Baseline refers to year 7 of the CARDIA cohort

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, explain how loss to follow-up was addressed
		(*e*) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) Summarise follow-up time (eg, average and total amount)
Outcome data	15*	Report numbers of outcome events or summary measures over time
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.