PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Long-Term Change in Alcohol Consumption Status and Variations in Fibrinogen Levels: The Coronary Artery Risk Development in Young Adults (CARDIA) Study
AUTHORS	Okwuosa, Tochi; Klein, Oana; Chan, Cheeling; Schreiner, Pamela; Liu, Kiang; Green, David

VERSION 1 - REVIEW

REVIEWER	R. Curtis Ellison, MD; Professor of Medicine & Public Health, Boston University School of Medicine, Boston, MA - USA. I get no research support or salary support from companies in the beverage industry, but travel to meetings or to give presentations are often covered by such.
REVIEW RETURNED	04-Apr-2013

RESULTS & CONCLUSIONS	the primary interest is a comparison of drinkers and quitters, not on race/sex differences (which should be presented but not the main outcome). Also, changes in fibrinogen for non-drinkers should be mentioned, but is not a key outcome.
GENERAL COMMENTS	This is a well-written paper carried out using well-described and appropriate analyses. The assessments of alcohol intake are satisfactory. Given the very limited amount of previous data available from prospective studies on subjects who have changed their alcohol intake during follow up, these are important results. The measurements of fibrinogen help determine if changes in clotting mechanisms may relate to the effects of alcohol on cardiovascular disease. Good data on potential confounders were available to the investigators. There is some question as to why the authors focus so much on sex/race differences, when the key outcome is the effect on fibrinogen according to stability or changes in alcohol intake, and the results are very similar for all ever-drinking groups. Further, the changes among non-drinkers (who generally differ in many ways from drinkers) is of interest, but not the key outcome. The main question being dealt with concerns the other three groups: “Do people who continue to drink or start to consume alcohol have different changes in fibrinogen from former drinkers who quit drinking?” Data presented in the Figure simply repeat data in Table 1. Further, if the non-drinkers are not included, the step-wise increase in fibrinogen for the “Became drinker,” “Stayed drinker,” and “Quit drinking” categories is the same for all groups, so an overall single figure (all groups combined, adjusted for race/sex) could be given

	instead of the present Figure. The reason why some people stop drinking could be very important, but the authors almost surely do not have good information on this. Still, it would be helpful to describe the health characteristics of stable drinkers, quitters, and beginning drinkers according to other diseases present: cardiovascular disease, cancer, dementia, etc.

REVIEWER	Licia Iacoviello, MD, PhD Head of laboratory of Genetic and Environmental Epidemiology. Research laboratories. Fondazione di Ricerca e Cura "Giovanni Paolo II". Catholic University. Campobasso. Rome I have no conflict of interest
REVIEW RETURNED	15-Apr-2013

THE STUDY	It is not clear how many participants have been finally considered for the present study The definition of drinking cathegories could allow misinterpretetion of the results. Methods for fibrinogen testing should be better specified The relation between alcohol consumption and cardiovascular disease should be mor precisely defined in the text. Selection of confounders should be better specified Interaction test should be performed.
RESULTS & CONCLUSIONS	o One of the most important limitations in interpreting results on alcohol consumption and health/disease is the (frequent) inclusion of former drinkers in the control group. Indeed, people generally quit drinking because of an upcoming disease status that can affect both the risk of cardiovascular disease and the levels of fibrinogen. The Authors not only do not take into account the effect(s) that including ex-drinkers in the control group may have on the observed differences in fibrinogen levels at year 7 in drinkers and non-drinkers, but also what are the reasons for drinking quitting at year 17.One would reasonably expect that quitters are subjects who developed a disease (probably at liver level) and this, beside alcohol, would be more than sufficient to justify a higher increase in fibrinogen levels. In the same way people who stayed drinkers or started to drink where possibly those in a better health status, to which lower increase in fibrinogen might reasonably correspond.
GENERAL COMMENTS	The Authors evaluated the long term changes of fibrinogen levels in relation to changes in alcohol consumption in the framework of the CARDIA study. They found that participants who became/stayed drinkers showed a lower increase in fibrinogen levels over 13 years, as compared to participants who never drunk. On the contrary, those who quitted drinking had a higher increase in fibrinogen. The Author conclude that their study provides new insight into the mechanism of moderate alcohol intake protection against cardiovascular disease. The study deals with an interesting topic and it is among the few that associated changes across years in alcohol status and fibrinogen levels. However some issues should be mentioned here that can affect the interpretation of the results. o One of the most important limitations in interpreting results on alcohol consumption and health/disease is the (frequent) inclusion of former drinkers in the control group. Indeed, people generally quit

	drinking because of an upcoming disease status that can affect both the risk of cardiovascular disease and the levels of fibrinogen. The Authors not only do not take into account the effect(s) that including ex-drinkers in the control group may have on the observed differences in fibrinogen levels at year 7 in drinkers and non-drinkers, but also what are the reasons for drinking quitting at year 17.One would reasonably expect that quitters are subjects who developed a disease (probably at liver level) and this, beside alcohol, would be more than sufficient to justify a higher increase in fibrinogen levels. In the same way people who stayed drinkers or started to drink where possibly those in a better health status, to which lower increase in fibrinogen might reasonably correspond. o Where people who could be identified as “occasional drinkers” included in the cohort? If so, in which cathegory were they classified? o Characteristics of subjects at year7 according to drinking status should be reported. o It is not clear how many participants have been finally considered for the present study; indeed in the method section they reported: “the final cohort included 2,548 participants (line 10), We included 2,520…. (line 14) and again 2,548 participants (results line 2). o Moreover the Authors state that 5,115 adults were initially included into the study and that 423 subjects were excluded for various reasons for the present analysis. Then the final cohort included for the analysis was 2,548. There should be something missing between the original and the present cohort. • How could fibrinogen have been measured in serum? It is measured in plasma where anticoagulation preserves fibrinogen consumption by clotting. o It is better to use “moderate alcohol” when referring to cardiovascular disease (Introduction, line 9 and thrughout all the manuscript) o The quality of figure 1 is quite low, please modify. o How confounders introduced in multivariable analysis where selected? o It would be interesting to calculate formal interactions for gender and race in the difference in fibrinongen increase across alcohol status changes.

VERSION 1 – AUTHOR RESPONSE

Reviewer: R. Curtis Ellison, MD; Professor of Medicine & Public Health, Boston University School of Medicine, Boston, MA - USA.

I get no research support or salary support from companies in the beverage industry, but travel to meetings or to give presentations are often covered by such.

As suggested below, the primary interest is a comparison of drinkers and quitters, not on race/sex differences (which should be presented but not the main outcome). Also, changes in fibrinogen for non-drinkers should be mentioned, but is not a key outcome.

This is a well-written paper carried out using well-described and appropriate analyses. The assessments of alcohol intake are satisfactory. Given the very limited amount of previous data available from prospective studies on subjects who have changed their alcohol intake during follow up, these are important results.

The measurements of fibrinogen help determine if changes in clotting mechanisms may relate to the

effects of alcohol on cardiovascular disease. Good data on potential confounders were available to the investigators.

There is some question as to why the authors focus so much on sex/race differences, when the key outcome is the effect on fibrinogen according to stability or changes in alcohol intake, and the results are very similar for all ever-drinking groups. CARDIA STUDY POPULATION WAS DESIGNED TO BE BALANCED (I.E., APPROXIMATELY EQUAL NUMBERS OF PARTICIPANTS) BY AGE (18-24 YEARS OR 25-30 YEARS), SEX, RACE (SELF-DEFINED: BLACK OR WHITE), AND EDUCATION (< HIGH SCHOOL OR > HIGH SCHOOL) WHEN RECRUITED AT BASELINE. THESE CATEGORIES WERE GATHERED TO CONTROL FOR CONFOUNDING IN RISK FACTOR IDENTIFICATION. IN ADDITION, WE FORMALLY TESTED FOR INTERACTIONS BETWEEN CHANGES IN FIBRINOGEN AND SEX-RACE; THE RESULTS SHOWED ONE OR MORE OF THE INTERACTION TERMS WERE SIGNIFICANT (P VALUE RANGE 0.01-0.30). THUS, IT IS JUSTIFIED TO STRATIFY THE STUDY POPULATION BY SEX-RACE.
Further, the changes among non-drinkers (who generally differ in many ways from drinkers) is of interest, but not the key outcome. The main question being dealt with concerns the other three groups: “Do people who continue to drink or start to consume alcohol have different changes in fibrinogen from former drinkers who quit drinking?” THE SHORT ANSWER IS YES (PLEASE SEE TABLE 1 BELOW). WE HAVE INCLUDED A STATEMENT TO THIS EFFECT IN THE RESULTS SECTION OF THE MANUSCRIPT.
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§
Alcohol use									
Continued non-drinker	124	71.9 (6.0)	69.9 (6.1) c	139	57.8 (5.4) a	56.2 (5.5) a	338	84.0 (4.4)	84.0 (4.6)	228	82.2 (4.9) c	82.7 (4.9)
Became drinker	39	66.3 (10.8)	68.4 (10.7)	84	61.5 (7.0) b	61.5 (7.0) b	78	62.1 (9.2)	62.8 (9.2)	108	54.3 (7.0) a	53.6 (6.9) a
Stayed drinker	191	67.7 (4.9) c	68.4 (4.9) c	415	72.3 (3.1) c	73.3 (3.2)	149	69.9 (6.7)	69.9 (6.9)	333	55.4 (4.0) a	56.2 (4.1) a
Quit drinking (Ref)	65	89.9 (8.3)	90.6 (8.2)	78	90.1 (7.2)	87.5 (7.3)	86	93.0 (8.8)	82.3 (8.8)	65	103.4 (9.0)	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.
aP <0.001, bP<0.01, cP<0.05 compared with the referent category of risk factor within sex/race.

Data presented in the Figure simply repeat data in Table 1. Further, if the non-drinkers are not included, the step-wise increase in fibrinogen for the “Became drinker,” “Stayed drinker,” and “Quit drinking” categories is the same for all groups, so an overall single figure (all groups combined, adjusted for race/sex) could be given instead of the present Figure. THANK YOU. WE HAVE AMENDED THE FIGURE TO INCLUDE PERTINENT DATA (THE NUMBER OF INDIVIDUALS IN EACH GROUP), AND HAVE REMOVED THAT PARTICULAR TABLE ALTOGETHER.
The reason why some people stop drinking could be very important, but the authors almost surely do not have good information on this. Still, it would be helpful to describe the health characteristics of

stable drinkers, quitters, and beginning drinkers according to other diseases present: cardiovascular disease, cancer, dementia, etc. WE HAVE NOW DESCRIBED THE HEALTH CHARACTERISTICS OF THE PARTICIPANTS BY ALCOHOL CONSUMPTION CATEGORY. THIS IS NOW LABELED 'TABLE 1' IN OUR MANUSCRIPT. THIS UNADJUSTED DATA SHOW THAT AT BASELINE (YEAR 7), THE CONTINUED NON-DRINKER POPULATION AND THOSE WHO QUIT DRINKING HAD SIGNIFICANTLY HIGHER PREVALENCE OF HIGH BLOOD PRESSURE – WHICH INCREASED AND REMAINED SIGNIFICANT BY YEAR 20 – COMPARED WITH THOSE WHO BECAME OR STAYED DRINKER BY FOLLOWUP. THOSE WHO QUIT DRINKING HAD SIGNIFICANTLY LOWER PREVALENCE OF DIABETES AT BASELINE, WHICH INCREASED (BUT NOT SIGNIFICANTLY) BY FOLLOWUP AT YEAR 20. OTHER ASSESSED CHARACTERISTICS WERE NOT SIGNIFICANTLY DIFFERENT BETWEEN THE ALCOHOL CONSUMPTION STATUS GROUPS.

Reviewer: Licia Iacoviello, MD, PhD
Head of laboratory of Genetic and Environmental Epidemiology. Research laboratories.
Fondazione di Ricerca e Cura "Giovanni Paolo II". Catholic University. Campobasso. Rome

I have no conflict of interest

It is not clear how many participants have been finally considered for the present study. THANK YOU FOR POINTING THIS OUT. WE INCLUDED 2520 PARTICIPANTS IN OUR STUDY. WE HAVE NOW MADE CORRECTIONS TO THE METHODS SECTION. The definition of drinking cathegories could allow misinterpretetion of the results. WE DO NOT UNDERSTAND THE REVEIWER'S COMMENTS. WE DO BELIEVE WE WERE AS SPECIFIC AS POSSIBLE IN DEFINING THE VARIOUS CATEGORIES OF THE ALCOHOL CONSUMPTION GROUPS IN THE METHODS SECTION OF OUR MANUSCRIPT, UNDER THE SECTION TITLED "ALCOHOL CONSUMPTION".
Methods for fibrinogen testing should be better specified. THANK YOU. THE METHODS SECTION TITLED "FIBRINOGEN MEASUREMENT" HAS BEEN MODIFIED TO BETTER DESCRIBE FIBRINOGEN TESTING.

The relation between alcohol consumption and cardiovascular disease should be more precisely defined in the text. THE DISCUSSION SECTION NOW CONTAINS INFORMATION REGARDING THE PHYSIOLOGY OF ALCOHOL AND CVD, AS WELL AS FIBRINOGEN AND CVD.

Selection of confounders should be better specified. Interaction test should be performed. AS STATED BELOW, WE FORMALLY TESTED THE INTERACTIONS BETWEEN CHANGES IN FIBRINOGEN AND SEX-RACE; THE RESULTS SHOWED ONE OR MORE OF THE INTERACTION TERMS WERE SIGNIFICANT (P VALUE RANGE 0.01-0.30).

The Authors evaluated the long term changes of fibrinogen levels in relation to changes in alcohol consumption in the framework of the CARDIA study. They found that participants who became/stayed drinkers showed a lower increase in fibrinogen levels over 13 years, as compared to participants who never drunk. On the contrary, those who quitted drinking had a higher increase in fibrinogen. The Author conclude that their study provides new insight into the mechanism of moderate alcohol intake protection against cardiovascular disease.
The study deals with an interesting topic and it is among the few that associated changes across years in alcohol status and fibrinogen levels.
However some issues should be mentioned here that can affect the interpretation of the results.

o One of the most important limitations in interpreting results on alcohol consumption and health/disease is the (frequent) inclusion of former drinkers in the control group. Indeed, people generally quit drinking because of an upcoming disease status that can affect both the risk of cardiovascular disease and the levels of fibrinogen. The Authors not only do not take into account the

effect(s) that including ex-drinkers in the control group may have on the observed differences in fibrinogen levels at year 7 in drinkers and non- drinkers, but also what are the reasons for drinking quitting at year 17. One would reasonably expect that quitters are subjects who developed a disease (probably at liver level) and this, beside alcohol, would be more than sufficient to justify a higher increase in fibrinogen levels. In the same way people who stayed drinkers or started to drink where possibly those in a better health status, to which lower increase in fibrinogen might reasonably correspond. PLEASE NOTE THAT THE EX-DRINKERS ARE NOT PART OF THE CONTROL GROUP; THEY ARE A GROUP OF THEIR OWN – THE QUIT DRINKING GROUP.
WE HAVE NOW INCLUDED A TABLE OF HEALTH CHARACTERISTICS AT BASELINE AND FOLLOWUP. WE DESCRIBED THE HEALTH CHARACTERISTICS OF THE PARTICIPANTS BY ALCOHOL CONSUMPTION CATEGORY (TABLE 1). THIS UNADJUSTED DATA SHOW THAT AT BASELINE (YEAR 7), THE CONTINUED NON-DRINKER POPULATION AND THOSE WHO QUIT DRINKING HAD SIGNIFICANTLY HIGHER PREVALENCE OF HIGH BLOOD PRESSURE – WHICH INCREASED AND REMAINED SIGNIFICANT BY YEAR 20 – COMPARED WITH THOSE WHO BECAME OR STAYED DRINKER BY FOLLOWUP. THOSE WHO QUIT DRINKING HAD SIGNIFICANTLY LOWER PREVALENCE OF DIABETES AT BASELINE, WHICH INCREASED (BUT NOT SIGNIFICANTLY) BY FOLLOWUP AT YEAR 20. INTERESTINGLY, OTHER ASSESSED CHARACTERISTICS (INCLUDING LIVER DISEASE, HEPATITIS, DIGESTIVE DISEASE AND CANCER) WERE NOT SIGNIFICANTLY DIFFERENT BETWEEN THE ALCOHOL CONSUMPTION STATUS GROUPS.

o Where people who could be identified as “occasional drinkers” included in the cohort? If so, in which category were they classified? OUR STUDY STATES THAT “CURRENT ALCOHOL DRINKERS WERE DEFINED AS INDIVIDUALS WHO DRANK ANY ALCOHOLIC BEVERAGES IN THE PAST YEAR.” THIS IS WHAT OUR CATEGORIES OF ALCOHOL USE WAS BASED ON.

o Characteristics of subjects at year7 according to drinking status should be reported. WE HAVE NOW INCLUDED A TABLE (TABLE 1) WITH THIS INFORMATION. WE HAVE ALSO INCLUDED A PARAGRAPH IN THE RESULTS SECTION DESCRIBING THIS TABLE.

o It is not clear how many participants have been finally considered for the present study; indeed in the method section they reported: “the final cohort included 2,548 participants (line 10), We included 2,520…. (line 14) and again 2,548 participants (results line 2). WE THANK THE REVIEWER FOR NOTING THIS DISCREPANCY. WE HAVE NOW MADE CORRECTIONS TO THE METHODS SECTION ACCORDINGLY.

o Moreover the Authors state that 5,115 adults were initially included into the study and that 423 subjects were excluded for various reasons for the present analysis. Then the final cohort included for the analysis was 2,548. There should be something missing between the original and the present cohort. THANK YOU. OUR ANSWER TO THIS QUESTION IS AS ABOVE.

• How could fibrinogen have been measured in serum? It is measured in plasma where anticoagulation preserves fibrinogen consumption by clotting. THANK YOU. FIBRINOGEN IS INDEED MEASURED IN PLASMA. WE HAVE MADE CORRECTIONS TO THE MANUSCRIPT ACCORDINGLY.

o It is better to use “moderate alcohol” when referring to cardiovascular disease (Introduction, line 9 and throughout all the manuscript). WE BELIEVE WE WERE THOROUGH IN SPECIFICALLY STATING THE THE RELATIONSHIP BETWEEN ‘MODERATE’ ALCOHOL CONSUMPTION AND CVD WITHIN THE TEXT. WE EDITED VERY LITTLE REGARDING THIS CONCEPT IN THE CURRENT EDITED MANUSCRIPT.

o The quality of figure 1 is quite low, please modify. WE THANK THE REVIEWER FOR THIS COMMENT. WE ARE NOT SURE WE UNDERSTAND THIS STATEMENT, OR HOW TO MODIFY THE FIGURE WHICH WE BELIEVE IS QUITE SELF-EXPLANATORY.

o How confounders introduced in multivariable analysis were selected? THESE ARE RISK FACTORS KNOWN TO BE ASSOCIATED CARDIOVASCULAR DISEASE AND FIBRINOGEN LEVELS IN OTHER STUDIES.

o It would be interesting to calculate formal interactions for gender and race in the difference in fibrinongen increase across alcohol status changes. WE FORMALLY TESTED THE INTERACTIONS BETWEEN CHANGES IN FIBRINOGEN AND SEX-RACE; THE RESULTS SHOWED ONE OR MORE OF THE INTERACTION TERMS WERE SIGNIFICANT (P VALUE RANGE 0.01-0.30).

VERSION 2 – REVIEW

REVIEWER	Licia Iacoviello, MD, PhD Laboratory Head. Catholic University. Campobasso. Italy
REVIEW RETURNED	19-May-2013

GENERAL COMMENTS	The authors answered to all my questions. The manuscript is now acceptable for publication.