Title: Plasma albumin and incident cardiovascular disease: results from the Copenhagen General Population Study and an updated meta-analysis

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ABSTRACT

Objective: We studied the association of plasma albumin with cardiovascular disease (CVD) and explored potential mechanisms behind the association in the Copenhagen General Population Study. We also performed a meta-analysis to summarize the association between plasma albumin and CVD in individuals without pre-existing CVD.

Approach and Results: We included 100,520 individuals without prior CVD with 8247 incident CVD events developed during a median follow-up of 8.5 years. Rates of CVD outcomes were calculated using Cox regression and Fine and Gray competing-risks regression. The association of plasma albumin and CVD was approximately linear and confounder adjustment had little influence on the effect estimates, except for some attenuation after C-reactive protein adjustment. In analyses according to subtypes of CVD events, the hazard ratios for each 10 g/L lower plasma albumin were 1.17 (95%: 1.08-1.28) for ischemic heart disease, 1.25 (1.09-1.43) for myocardial infarction, 1.37 (1.21-1.54) for any stroke, and 1.46 (1.28-1.68) for ischemic stroke. In the meta-analysis, we combined estimates from prospective and nested case-control studies investigating the association of plasma albumin with CVD. The meta-analysis included 14 studies with 150,652 individuals (12 studies reported events totalling 11,872). The risk ratio for a CVD event per 10 g/L lower plasma albumin was 1.96 (1.43-2.68) in previous studies and 1.85 (1.39-2.47) including our study with 57% weight in the meta-analysis.

Conclusions: There is a robust, independent association of low plasma albumin with CVD, partly explained by plasma albumin as a negative acute phase reactant.

Key words: albumin, biomarker, cardiovascular disease, myocardial infarction, mortality

Abbreviations

AIC: Akaike information criterion

ALT: Alanine aminotransferase

BMI: Body mass index

CGPS: Copenhagen General Population Study

CVD: Cardiovascular disease

eGFR: Estimated glomerular filtration rate

HDL: High-density lipoprotein

ICD: International classification of disease

hsCRP: High sensitivity CRP

MI: Myocardial infarction

NOS: Newcastle-Ottawa Scale

PRISMA: Preferred Reporting Items for Systematic Review and Meta-analyses

Introduction

Albumin is the most abundant plasma protein with a number of biological functions including maintenance of colloidal osmotic pressure,[1] binding of endo- and exogenous substances[2], and anti-thrombotic effects.[3] Plasma albumin often declines in acute and chronic disease and provides strong, independent information about prognosis in these diseases.[4–6] The vast majority of the literature on plasma albumin and disease has focused on individuals with preexisting disease, primarily chronic liver disease and chronic kidney disease.[7,8] While many hypothetical mechanisms have been suggested to link plasma albumin with cardiovascular disease (CVD),[2,9] there is little evidence form human studies. Two mechanisms seem to be directly relatable to pathogenesis of CVD and regulation of plasma albumin; that is, hypertriglyceridemia[10] and chronic inflammation[11] seems to be causally linked to development of CVD and plasma albumin is known to be an important carrier of fatty acids[12] and inflammation is associated with hypoalbuminemia. However, few studies have investigated the association of plasma albumin with CVD in the general population or explored possible mechanisms of this association.[13]

We tested the hypothesis that plasma albumin is associated with risk of CVD in the general population. For this purpose, we tested the association of plasma albumin with CVD in over 100,000 individuals from the Copenhagen General Population Study and performed an updated and comprehensive meta-analysis of the association between plasma albumin and CVD in individuals without preexisting CVD. In the former analyses, we investigated possible mechanisms by epidemiological techniques, that is, interaction and stratified analyses of plasma albumin using triglycerides or C-reactive protein as well as genetic proxies for triglycerides (in *APOA5* and *LPL*) and inflammation (in *CRP* and *IL6R*) to investigate possible causality in the association of triglycerides or

inflammation with plasma albumin. Genetic proxies are less prone to confounding and largely free of bias due to reverse causation enabling analyses on causality[14].

Material and Methods

Study population

The Copenhagen General Population Study (CGPS) is a prospective cohort study initiated in 2003 with ongoing enrollment. Individuals aged 20 to 100 were randomly invited from the national Danish Central Person Register[15] to represent the white Danish general population. Participants completed a questionnaire, reviewed together with an examiner at the day of attendance, underwent a physical examination, and provided blood for plasma measurements. The participation rate in the CGPS was 43%. The dataset included considered 107,899 white individuals of Danish descent with plasma albumin measurements (missing in 1129 individuals, **Supplementary Table S1)**. Informed written consent was obtained from all individuals, and institutional review boards and Danish ethics committees approved the study. The study complied with the Declaration of Helsinki.

Exposure and covariates

Plasma albumin was measured using a bromcresol purple assay on freshly collected samples on the day of examination (Konelab, Thermo Fisher Scientific, Helsinki, Finland). Self-reported information on smoking (never, former, current, daily amount of tobacco, and years of smoking) and alcohol intake were collected. The following covariates were measured on the day of attendance/baseline: systolic and diastolic blood pressure, body mass index (BMI), calculated from measured weight in kilograms divided by measured height in meters squared; plasma total cholesterol, high-density lipoprotein (HDL) cholesterol, alanine aminotransferase (ALT), hemoglobin, thrombocytes, high

sensitivity CRP (hsCRP) and creatinine, measured using standard hospital assays; and estimated glomerular filtration rate (eGFR), calculated using the 2009 CKD-EPI Creatinine Equation.[16] Preexisting diabetes was defined as self-reported diabetes of any type, a hospital diagnosis of diabetes prior to examination (ICD8: 249-250, ICD10: E10, E11, E13, E14), non-fasting plasma glucose >11mmol/L at the examination or use of anti-diabetic medication. Genotyping was carried out using TaqMan assays as described previously (10.1161/CIRCULATIONAHA.113.003008, 10.1093/eurheartj/ehs431).

Outcome

Any diagnoses of ischemic heart disease (ICD8: 410-414; ICD10: I20-I25), myocardial infarction (ICD8 410 and ICD10 I21-22), stroke (any type [ICD8 432-435 and ICD10 I60-I64] and ischemic stroke separately [ICD8 433-434 and ICD10 I63]) from 1977 until April 2018 were obtained from the Danish National Patient Registry[17] and the Danish Register of Causes of Death.[18] For individuals with registered cerebrovascular disease, records from general practitioners and hospital records were requested, and the diagnosis of stroke was validated by two independent medical doctors, blinded to the test results as described previously.[19] To maximize power, we considered a single composite endpoint that included all outcomes (with the date of occurrence of the first event being taken as the date of the composite event); however, analyses were also performed on each outcome separately.

Statistical analyses

We used Stata v.13.1 and R version 3.5.3 for analyses in the CGPS. P-values for trends across three groups were estimated using Cuzick's non-parametric trend test. All reported p-values were two-

sided. Plasma albumin was divided into four predefined groups according to previous literature, and additional analyses using spline models were carried out to explore the association of extreme concentrations of plasma albumin with CVD risk. The data for covariates were >98% complete and missing values were imputed using multiple imputation by chained equations with fully conditional specification of prediction equations for multivariable adjustment to obtain a complete dataset; however, if individuals with any missing data were excluded, results were similar to those presented.

First, to examine the association between plasma albumin and the composite endpoint, as well as outcomes separately, we used Cox proportional hazards regression models with entry at examination and age as the time scale to estimate hazard ratios with 95% confidence intervals (CI). Individuals who emigrated (n=450) or died during follow-up without an event (n=6409) were censored at their emigration or death dates. Multivariable adjustment was for covariates that could confound the association between plasma albumin and outcomes and were chosen *a priori* (see exposures and covariates). Test for proportionality of hazards over time was performed using graphical methods and residuals; no major violations were observed. Furthermore, we performed multivariable adjusted Cox regression using restricted cubic splines for graphical representation of the associations. Knots were chosen based on Akaike information criterion (AIC).

Second, we investigated the associations using similar Cox regressions for each major outcome separately to identify any potential disease-specific patterns. Also, we carried out *a priori* defined stratified and interactions analyses since previous studies indicated that the associations may differ according to age, sex, or smoking status.[20–22] For exploratory analyses into possible mechanisms, we also carried out analyses stratified according to plasma triglycerides and plasma C-reactive protein as well as analyses of the association of plasma triglycerides and plasma C-reactive protein with CVD

stratified according to plasma albumin. These models were kept simple to extract information on the effect of adjustment on risk estimates and avoid over-adjustment and possible collider stratification bias.

Third, since Cox regression may lead to biased effect estimates in the presence of a competing risk such as death or emigration, we depicted cumulative incidence by using multivariable adjusted competing-risks survival regression with the method of Fine and Gray.[23]

Fourth, in order to assess the robustness of our analyses to potential unmeasured confounding we determined E values of our fully adjusted association estimate. A large E value implies that considerable unmeasured confounding is be needed to explain away the association[24].

Fifth, we tested the added value of plasma albumin in the Framingham risk score for CVD[25] using Harrel's C-index as a global concordance index,[26] continuous net reclassification index (NRI(>0), here NRI), and integrated discrimination index (IDI).[27] NRI is defined as NRI = P(up|event)−P(down|event) + P(down|nonevent)−P(up|nonevent) and can be understood as the appropriate change in probabilities assigned to cases and non-cases (theoretical range −2 to +2).[28] IDI can be defined as IDI=$(IS_{new}-IS_{old})-(IP_{new}-IP_{old})$, where IS is the integral of sensitivity over all possible cut-off values from the (0, 1) interval and IP is the corresponding integral of 1-specificity.[28] IDI increases with increased separation in probabilities assigned to cases versus non-cases. In all these models, plasma albumin was used as a continuous variable.

Lastly, we carried out additional exploratory analyses investigating possible mechanisms connecting plasma albumin with cardiovascular disease using genetic proxies for plasma triglycerides and C-reactive protein/inflammation. We used multivariable linear regression models adjusted for age, sex, and month and year of blood sampling. Genetic analyses are less prone to confounding and largely free of bias due to reverse causation.[14]

Meta-analysis

The meta-analysis was completed following the Preferred Reporting Items for Systematic Review and Meta-analyses (PRISMA) checklist.[29] Articles were identified by an electronic search using PubMed/MEDLINE from inception to May 2018. The following search string was used: ("albumin") AND ("cardiovascular disease" OR "CVD" OR "stroke" OR "myocardial infarction" OR "MI" OR "AMI") AND ("follow*" OR "prospective" OR "longitudinal" OR "cohort" OR "case-control"). We additionally performed a search using Embase Ovid using a similar search string as detailed in the supplementary material. Articles were eligible if i) the participants were from the general population; ii) the design was a longitudinal or a nested case-control study; ii) the exposure of interest was plasma albumin measurement (either categorized or continuous); iii) the outcome was a CVD event and/or CVD mortality; and iv) an effect estimate with a 95% confidence interval that was at least adjusted for age was reported. All included articles were finally examined for references and cross-references and citations were further assessed using the citation database Scopus. Two reviewers (AR and TD) extracted data based on the inclusion criteria outlined above. Any disagreement over eligibility was resolved over discussion with a third reviewer (SA). The risk of study bias was assessed using the Newcastle-Ottawa Scale (NOS) for nonrandomised studies in meta-analyses,[30] and a score of six was considered to be of good quality. The meta-analysis was pre-registered in the PROSPERO International Registry (CRD42018095796). We used both a random and a fixed effect model to combine the estimates with the same measures of association for a common risk ratio (RR_{pooled}). Further details on statistical analysis, including calculation of publication bias, inter-study heterogeneity and moderators, can be found in the supplementary material.

Results

Characteristics of the Copenhagen General Study Population

Among 107,899 individuals with plasma albumin measurements, 7379 had experienced the composite CVD outcome before baseline examination, leaving 100,520 individuals for prospective analyses. Baseline characteristics of the study population are presented in **Table 1**. Median follow-up was 8.5 years (range: 0.003-14.3 years) for the composite CVD outcome and 8247 individuals had an event. Lower plasma albumin at baseline was associated with several risk factors for CVD, e.g. female sex, being non-smoker, lower alcohol consumption, lower systolic blood pressure, lower BMI, diabetes, and lower non-HDL cholesterol.

Plasma albumin and risk of cardiovascular disease in the Copenhagen General Population Study

The association of plasma albumin with the CVD outcome was approximately linear with higher risk of the composite CVD outcome with lower plasma albumin (**Figure 1**). Adjustments for potential confounders had little influence on the effect estimates. We carried out an additional analysis to investigate the association of extreme values with risk of CVD showing an almost a linear increase in CVD rate with lower plasma albumin, especially below ~ 39 g/L (**Supplementary Figure S1**).

The multivariable adjusted hazard ratio was 1.22 (95% confidence interval: 1.12-1.32) for plasma albumin <35 g/L versus plasma albumin 40-44.9 g/L and 1.23 (1.14-1.32) for each 10 g/L lower plasma albumin (**Figure 1** and **Figure 2**). In analyses according to subtypes of CVD events, the hazard ratios for each 10 g/L lower plasma albumin were 1.17 (1.08-1.28) for ischemic heart disease, 1.25 (1.09-1.43) for myocardial infarction, 1.37 (1.21-1.54) for any stroke, and 1.46 (1.28-1.68) for ischemic stroke (**Figure 2**).

We further investigated the potential contribution of plasma albumin to performance of the Framingham risk score for CVD. After recalibration to our population, the addition of plasma albumin slightly improved Harrel's c-statistic by 0.002 (0.001-0.003) from c=0.685. NRI and IDI also showed a minor improvement in model performance after addition of plasma albumin to the Framingham risk score for CVD, that is, IDI was 0.003 (0.002-0.004) and continuous NRI was 0.12 (0.11-0.14).

Sensitivity analyses

In the Copenhagen General Population Study, we carried out analyses to account for potential biases due to the competing risk of death and found similar results as those derived from a Cox regression (**Figure S2**).

In further analyses, we tested whether estimates differed according to age, sex, or smoking status as well as the effect of excluding the first two years of follow-up. We found that estimates may be stronger among those aged <60 years while estimates were similar in strata of sex and smoking status (**Supplementary Figure S3**). Also, excluding the first two years of follow-up produced similar results as the main analyses (**Supplementary Figure S4**).

To estimate robustness of risk estimates to confounding, we calculated E-values for plasma albumin categories. Additionally, we provide similar estimates for non-HDL cholesterol, a causal risk factor for CVD, in categories corresponding to those used for plasma albumin. That is, <35 g/L plasma albumin corresponds to the lowest decile; thus, we used estimates for the top decile of non-HDL cholesterol for context using <3.6 mmol/L as refence group. For an unmeasured confounder to shift the observed estimate of HR = 1.22 to an estimate of HR = 1.00, the unmeasured confounder should be associated with both plasma albumin <35g/L and the combined CVD outcome by a risk ratio of

1.7 each. Corresponding estimates for non-HDL cholesterol would be a shift from HR = 1.15 to 1.00 and a risk ratio of 1.6 fold for non-HDL cholesterol>5.4 mmol/L and the combined CVD outcome each.

Exploratory analyses of potential biological pathways

As mentioned previously, possible mechanisms driving the associations of plasma albumin with CVD could be transport of fatty acids by albumin and downregulation of plasma albumin by inflammation. If this is the case, we would expect: 1) an association of plasma albumin with plasma triglycerides and C-reactive protein (**Figure 3A**); 2) if triglycerides or C-reactive protein causally regulate plasma albumin concentration, we would expect genetic proxies for these variables to affect plasma albumin concentration (**Figure 3B**); and 3) attenuation of risk estimates for CVD by plasma albumin when adjusting for triglycerides and C-reactive protein or an interaction of plasma albumin with these variables on risk of CVD (**Figure 3C+D**).

We observed higher plasma C-reactive protein and lower plasma triglycerides with lower plasma albumin (Figure 4A). In genetic analyses there were no consistent associations of genetic proxies for triglycerides and C-reactive protein/inflammation with plasma albumin; however, *IL6R* rs4537545 as a marker of higher inflammation there was a trend toward lower plasma albumin (Figure 4B). It is puzzling why genetically higher triglycerides through *APOA5* rs651821 lead to lower plasma albumin. However, we note that the genotype leads to higher C-reactive making the pattern consistent with that seen for *IL6R* rs4537545.

In Figure 4C, adjustment for C-reactive protein (but not triglycerides) attenuated the hazard ratio for the association of plasma albumin with CVD (1.24 (1.16-1.33 versus 1.14 (1.06-1.22)). Lastly, in analyses stratified by triglycerides or C-reactive protein, estimates for the association of plasma

albumin with CVD appeared to be lowest in those with C-reactive protein below the median (≤1.5 mg/L) and thus no sign of low-grade inflammation. The associations of plasma triglycerides and C-reactive protein with CVD were similar across strata of plasma albumin (dichotomized at the median, **Supplementary Figure S5**).

Meta-analysis

The search yielded a total of 1768 studies. We excluded 1702 studies after screening the titles and abstracts. The remaining 66 studies were retrieved for a detailed full-text evaluation; of these, 19 studies were included (**Supplementary Table S2**). Statistical assessment of heterogeneity, bias and effect modifiers can be found as supplementary material (**Supplementary Table S3-S4, and Figure S6**). One study did not provide information to compute a continuous estimate;[31] thus, 18 studies were included. We calculated a combined estimate for studies reporting CVD events (**Figure 4**) and CVD mortality (**Supplementary Figure S7**). A total of 14 studies with 150,652 individuals assessed the association between plasma albumin and CVD events (12 studies reported events totalling 11,872). RR_{pooled} (95% CI) obtained by a random effect model was 1.96 (1.43-2.68) for CVD per 10 g/L lower plasma albumin in former studies and 1.85 (1.39-2.47) including our study in the meta-analysis (**Figure 5**). These numbers were 1.37 (1.26-1.49) and 1.29 (1.22-1.36) in a fixed effect model, which highlight the 57% statistical weight of the present study. To investigate whether the metanalysis was reproducible with an alternative outcome, we carried out analyses using CVD mortality as an outcome and results were similar (**Supplementary Figure S7)**.

Discussion

We found an inverse association between plasma albumin and CVD in our prospective study of more than 100,000 individuals, partly explained by albumin as a negative acute phase reactant. Results from our study, which is the largest study examining the association between plasma albumin and CVD to date, representing 57% of statistical weight in fixed effect models, are consistent in direction with results from our meta-analysis of previous studies, although the combined effect estimate was changed slightly (from RR_{pooled} 1.96 in previous studies to 1.85 including our study). We provide novel data on several aspects of this association. First, due to the size of our study, we were also able to provide a graphical representation of the association between a wide spectrum of plasma albumin levels and hazards of CVD as well as robust data on subtypes of CVD within the same cohort. Second, we show exploratory analyses into possible mechanisms explaining this association, suggesting that at least part of the inverse association between plasma albumin and CVD can be explained by plasma albumin as a negative acute phase reactant. Third, we show data on performance of the Framingham risk score for CVD after addition of plasma albumin, which only show minor improvements on its performance. Fourth, the robustness of the association was shown using analyses accounting for competing risk of death and un-measured confounding. The association was approximately linear and consistent across CVD subtypes and was robust accounting for different forms of bias.

There are several plausible biological mechanisms that could explain our findings. While plasma albumin has many roles, two mechanisms seem to be directly relatable to pathogenesis of cardiovascular disease and regulation of plasma albumin, as randomized intervention trials have shown that hypertriglyceridemia[10] and inflammation[11] seems to be causally linked to development of cardiovascular disease. First, as plasma albumin binds fatty acids (2-6 moles of unesterified fatty acids per albumin molecule)[12], it may be hypothesized that plasma albumin modifies the association

of fatty acids and triglycerides with CVD. However, the association of plasma albumin with triglycerides was modest. Also, we were not able to show any interaction of plasma triglycerides with plasma albumin on risk of CVD. Second, inflammation is known to be associated with lower plasma albumin. Interestingly, the only adjustment that seemed to attenuate the risk estimate of plasma albumin with CVD was inclusion of C-reactive protein while those with C-reactive below the median had the smallest risk estimate. Also, in observational analyses lower plasma albumin was associated with higher C-reactive protein. Collectively, our analyses suggest that triglycerides and inflammation do not affect plasma albumin causally, but lower plasma albumin is possibly an epiphenomenon to inflammation suggesting that at least part of the inverse association between plasma albumin and cardiovascular disease can be explained by albumin as a negative acute phase reactant. A possible mechanism, that could explain the part of the findings not explained by the acute phase response, is that lower plasma albumin reflects increased vascular permeability in inflammatory processes, which is not captured by C-reactive protein, as fluid redistribution to the interstitium can quickly and dramatically change plasma albumin concentrations[3,6].

Other mechanism of plasma albumin may include its functions as an antioxidant, a carrier of several other types of molecules, and, possibly, a regulator of endothelial permeability. Plasma albumin scavenge free reactive oxygen and nitrogen species,[2,22] which are thought to cause CVD through endothelial dysfunction, inflammation and atherosclerosis.[,23] Also, plasma albumin binds eicosanoids and nitric oxide that may help regulate vascular tone and inhibit platelet aggregation that are intimately involved in the development of atherosclerosis and blood clots.[8,33] Finally, plasma albumin helps maintain endothelial permeability via interactions with interstitial matrix,[34]. Our study was not designed to investigate all potential mechanisms, and our results may have been confounded by sub-clinical disease, e.g. subclinical non-alcoholic fatty liver disease. Such diseases

may be associated with decreased production, or increased loss, of plasma albumin, and at the same time be associated with an increased risk of CVD, e.g. trough dyslipidemia, alterations in insulin resistance, and inflammation.[35,36]

Whether plasma albumin is causally associated with CVD or not, it may be a useful biomarker for risk stratification. We are not aware of any studies trying to include plasma albumin into existing CVD prognostic scores. Thus, we assessed whether plasma albumin would provide added information when included in the Framingham risk score. We found a minor improvement in prediction by including plasma albumin with the risk score; however, the improvements are probably too small to be of clinical significance.

Compared to most previous studies our cohort study has some advantages, that is, a larger sample size, more events, and several subtypes of CVD which could be investigated in the same cohort. Given the large sample size we were able to detect an approximately linear increase in CVD rate with decreasing plasma albumin, starting well within the reference range. Additionally, given that some of the studies were done several decades ago, most were not able to adjust for modern biomarkers of CVD such as C-reactive protein measured using a high sensitivity method, which we did. Some previous studies have shown an interaction of age and smoking with plasma albumin on risk of CVD. Among three studies that stratified their results by smoking status,[21,22,37] the association of plasma albumin with CVD was strongest in current smokers, while other smaller studies have indicated that the association may be strongest among younger individuals.[38,39] In the present study, there was some indication of effect modification by age but no convincing evidence of interaction was found for smoking status.

Limitations of the present cohort study and meta-analyses should also be considered. First, a limited number of studies have been published in a general population setting and are included in the present meta-analysis. Thus, the overall sample size was rather small, that is, before inclusion of our own study, and showed some evidence of publication bias. Second, in addition to the present study, most prior studies were conducted in the United States or Europe in primarily elderly populations of which many included men only, which limits generalizability to other geographic settings. Third, we cannot escape the limitations of observational studies such as the potential for residual confounding and reverse causation. Reduced plasma albumin is associated with several chronic diseases and risk factors for CVD,[9,27,30] which could confound our results if not adequately adjusted for. However, in the present cohort study we did adjust for major known risk factors of CVD, such as high blood pressure, smoking, cholesterol and low-grade inflammation, and additional biomarkers for other chronic diseases, e.g. ALT, eGFR, hemoglobin, and thrombocytes.

Our findings have some implications for future studies. First, studies are needed to investigate the association of plasma albumin with secondary CVD events. Second, as this and a few previous studies suggest, the association is strongest among younger individuals, perhaps studies directed towards early CVD events should investigate the possible contribution of plasma albumin to risk stratification in this subgroup. Finally, randomized intervention trials (e.g. anti-inflammatory studies) or genetic studies are required before any firm conclusions can be drawn regarding causality and further recommendation can be made regarding clinical use of these findings. However, designing proper and safe interventions to increase plasma albumin may be difficult and genetic variants affecting plasma albumin concentration will likely have pleiotropic effects as albumin has multiple functions.

In conclusion, there is a robust, independent association of low plasma albumin with CVD, partly explained by plasma albumin as a negative acute phase reactant.

Acknowledgments

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Highlights

- Plasma albumin is inversely associated with cardiovascular disease in The Copenhagen General Population Study consistent with our meta-analysis of previous studies reporting on this association.

- This association seems to be consistent across different cardiovascular disease outcomes and robust after adjustment for traditional cardiovascular risk factors.

- Our mechanistic explorative analyses observed that the inverse association between plasma albumin and cardiovascular disease was attenuated after adjustment for C-reactive protein, suggesting that at least part of the association can be explained by albumin as a negative acute phase reactant.

Figure legends

Figure 1. Association of plasma albumin with cardiovascular disease. Cardiovascular disease (CVD) was a combination of all CVD outcomes. All results were based on Cox regression models A: According to plasma albumin in groups in 3 different adjusted models for potential confounders. B: Spline models with identical adjustments on a continuous scale (graphs truncated at 1st and 99th percentile to enhance readability). BMI=Body mass index, HDL = high-density lipoprotein, hsCRP = C-reactive protein (high sensitivity assay), ALT = alanine aminotransferase, eGFR = estimated glomerular filtration rate, CI: confidence interval.

Figure 2. Association of plasma albumin with individual cardiovascular disease outcomes. Cox regression models adjusted for age, sex, smoking status, alcohol consumption, systolic blood pressure, BMI, diabetes, HDL cholesterol, non-HDL cholesterol, hsCRP, ALT, eGFR, hemoglobin, and thrombocytes. BMI=Body mass index, HDL = high-density lipoprotein, hsCRP = C-reactive protein (high sensitivity assay), ALT = alanine aminotransferase, eGFR = estimated glomerular filtration rate, CI: confidence interval.

Figure 3. Exploratory analyses into possible mechanisms explaining the association of plasma albumin with cardiovascular disease. A) Association of plasma albumin in percentiles with plasma triglycerides and C-reactive protein. Plasma albumin was used in percentiles to increase power for extreme groups. B) Association of genotypes strongly associated with plasma triglycerides or C-reactive protein with plasma albumin (N=99597). Genotypes in different genes were used to reflect different pathways, e.g. IL6R genotype reflects upstream regulation of inflammation and CRP genotypes reflect synthesis of C-reactive protein directly. C) Additional adjustment for plasma

triglycerides or C-reactive protein in age and sex adjusted models for the association of plasma albumin with CVD. D) Analyses age and sex adjusted models for the association of plasma albumin with CVD stratified according plasma triglycerides or C-reactive protein. hsCRP = C-reactive protein (high sensitivity assay), CI: confidence interval.

Figure 4: Flow diagram of study inclusion.

Figure 5. Forest plot of meta-analysis showing relative risk and 95% confidence interval of cardiovascular disease per 10 g/L lower plasma albumin. Weights were calculated using the fixed effect model. See Table 2 for an overview of studies and references. FE: fixed effect, RE: Random effect, CI: confidence interval

Table 1. Baseline characteristics in the Copenhagen General Population Study according to plasma albumin.

	Plasma albumin, g/L				
	<35 N=10862	35-39.9 N=53018	40-44.9 N=37633	>=45 N=6386	P for trend*
Plasma albumin, g/L	33.8 (32.7-34.4)	37.8 (36.6-38.9)	41.7 (40.8-42.9)	46.3 (45.5-47.5)	
Age, years	65.6 (52.1-74.2)	60.1 (49.6-68.7)	55.5 (46.8-64.2)	50.8 (43.4-59.1)	<0.0001
Men, N (%)	3791 (35)	22077 (42)	19013 (51)	3639 (57)	<0.0001
Current smoker, N (%)	1615 (15)	8534 (16)	6974 (19)	1365 (21)	<0.0001
Alcohol consumption, g/week	84 (36-168)	96 (36-168)	96 (36-180)	108 (48-192)	<0.0001
Systolic blood pressure, mmHg	137 (124-152)	140 (125-154)	140 (128-156)	140 (128-155)	<0.0001
Body mass index, kg/m2	25.3 (22.9-28.3)	25.5 (23.2-28.4)	25.7 (23.3-28.5)	25.4 (23.1-28.1)	<0.0001
Diabetes Mellitus, N (%)	545 (5)	2353 (4)	1485 (4)	214 (3)	<0.0001
HDL cholesterol, mmol/L	1.6 (1.3-2)	1.6 (1.2-1.9)	1.5 (1.2-1.9)	1.6 (1.3-2)	0.06
Non-HDL cholesterol, mmol/L	3.6 (3-4.4)	3.8 (3.1-4.6)	4 (3.3-4.8)	4.1 (3.3-4.9)	<0.0001
Plasma hsCRP, mg/L	1.8 (1.1-3.9)	1.4 (1-2.3)	1.3 (0.9-2)	1.2 (0.6-1.8)	<0.0001
Plasma ALT, U/L	18 (14-23)	19 (15-26)	21 (16-29)	23 (17-33)	<0.0001
Estimated glomerular filtration rate, mL/min	78.6 (67.1-89)	79.9 (69.1-90.4)	81 (70.4-91.8)	82 (72.2-92.7)	<0.0001
Whole Blood Hemoglobin, mmol/L	8.4 (7.9-8.9)	8.7 (8.2-9.2)	8.9 (8.4-9.4)	9.1 (8.6-9.6)	<0.0001
Whole Blood Thrombocytes, 10^9/L	273 (230-324)	270 (231-316)	270 (232-314)	269 (231-312)	<0.0001
Incident events CVD, no.	954	3987	2859	447	
Follow-up time, person-years	66684	385323	325506	66557	

Values for continuous variables are shown as median (interquartile range). *Calculated using Cuzick's trend test.

HDL = high-density lipoprotein, ALT = alanine aminotransferase, eGFR = estimated glomerular filtration rate (CKD-EPI equation), hsCRP = C-reactive protein (high sensitivity assay), CVD = combined cardiovascular disease outcome (ischemic heart disease and any stroke).