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Large-scale genetic analyses in an understudied disease

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Self-reported alcohol consumption, carbohydrate deficient transferrin and risk of cardiovascular disease: The PREVEND prospective cohort study

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ABSTRACT

Background: Self-reported alcohol consumption is an established risk factor for cardiovascular disease (CVD). Carbohydrate deficient transferrin (CDT) is an established objective marker of excessive alcohol consumption, but data on its prospective association with CVD are lacking. We aimed to evaluate the associations of self-reported alcohol consumption and CDT (expressed as %CDT, a more reliable marker than absolute CDT levels) with CVD risk.

Materials and methods: In the PREVEND prospective study of 5,206 participants (mean age, 53 years; 47.7% males), alcohol consumption by self-reports, absolute CDT measured using the Siemens nephelometric assay and %CDT calculated as the percentage of total transferrin concentrations, were assessed at baseline. Alcohol consumption was classified into 5 categories: abstention (reference), light, light–moderate, moderate and heavy alcohol consumption. Hazard ratios (HRs) (95% confidence intervals [CI]) for first CVD events were estimated.

Results: Mean (SD) of %CDT was 1.59 (0.54) %. During a median follow-up of 8.3 years, 326 first CVD events were recorded. Compared with abstainers, the multivariable-adjusted HRs (95% CIs) of CVD for light, light–moderate, moderate and heavy alcohol consumption were 0.66 (0.46–0.95), 0.83 (0.62–1.11), 0.83 (0.61–1.14) and 0.80 (0.48–1.36), respectively. Light alcohol consumption was associated with reduced coronary heart disease risk 0.62 (0.40–0.96), whereas light-moderate alcohol consumption was associated with reduced stroke risk 0.45 (0.24–0.83). The association of %CDT with CVD risk was not significant.

Conclusions: Our findings confirm the established association between self-reported light to moderate alcohol consumption and reduced CVD risk. However, %CDT within the normal reference range may not be a risk indicator for CVD.

1. Introduction

Cardiovascular disease (CVD), which accounts for over 17 million deaths each year, is the leading cause of mortality in the world [1]. By 2030, the World Health Organization estimates that almost 23.6 million people will die from CVD [2]. Major risk factors for CVD include blood lipids, blood pressure, a history of diabetes, smoking status as well as alcohol consumption [3]. Alcohol consumption is an established risk

Abbreviations: AST, aspartate aminotransferase; apoA-I, apolipoprotein, apolipoprotein A-I; BMI, body mass index; CI, confidence interval (CI); CDT, carbohydrate deficient transferrin; CHD, coronary heart disease; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; GGT, gamma-glutamyltransferase; HDL-C, high-density lipoprotein cholesterol; HR, hazard ratio; hsCRP, high sensitivity C-reactive protein; IQR, interquartile range; MCV, mean cell volume; %CDT, percent CDT; PON-1, paraoxonase-1; PREVEND, Prevention of Renal and Vascular End-stage Disease; SD, standard deviation; SBP, systolic blood pressure.

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factor for several chronic diseases including CVD [4,5]. A J-shaped relationship has consistently been described between alcohol consumption and CVD in several epidemiological studies, with light to moderate alcohol consumption being associated with lower vascular risk and heavy consumption associated with increased vascular risk [5,6]. Several other studies have challenged the J-shaped relationship [6–8] giving rise to ambiguities regarding drinking risk thresholds, limits for safe drinking and varying alcohol consumption guidelines across the globe [8]. This is also complicated by the fact that data on alcohol consumption in these studies have mostly depended on self-reports. The use of self-reported alcohol consumption potentially leads to the underestimation of the biological effects of alcohol exposure due to underreporting of consumption [9]. Objective biological markers of alcohol consumption are therefore needed to quantify the risk of CVD and other chronic diseases related to alcohol exposure.

Carbohydrate deficient transferrin (CDT) is an established marker that has been shown to be more specific than other widely used biochemical tests such as gamma-glutamyltransferase (GGT), aspartate aminotransferase (AST) or mean cell volume (MCV), for detecting excessive alcohol consumption [10]. Its advantage is that it is formed by a direct effect of alcohol; it originates from disturbances in the glycosylation of transferrin resulting from excessive drinking. Chronic alcohol consumption alters the glycosylation of many serum glycoproteins, resulting in the formation of abnormal isoforms that are responsible for microheterogeneity of these glycoproteins [11]. Carbohydrate deficient transferrin is the isoform of transferrin which is deficient in sialic acid residues [12]. The other biomarkers are indirect markers of alcohol consumption, as they do not directly represent metabolites of alcohol, but merely express the influence of alcohol on the liver. Carbohydrate deficient transferrin has been used for long term monitoring of early detection of relapse drinking during rehabilitation and in the assessment for reinstating driver licenses [13]. Given that CDT is known to vary with sex and age and to compensate for variations in the total transferrin concentration in various conditions (e.g., iron deficiency, iron overload) [14], the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Working Group has proposed percent CDT (%CDT) (i.e., CDT as a percentage of total transferrin) as the preferred method of reporting, as it is superior to absolute CDT as an alcohol biomarker [15,16]. Data on the prospective association between CDT and CVD are lacking. In a study that evaluated the cross-sectional associations of CDT, GGT and self-reported drinking with prevalent coronary heart disease (CHD), Jousilahti and colleagues reported CDT levels to be inversely and GGT levels to be positively associated with CHD risk [17]. The authors postulated that these relationships may underlie the curvilinear dose-response relationship between alcohol consumption and CHD risk. We have recently shown in the Prevention of Renal and Vascular End-stage Disease (PREVEND) prospective cohort study that increased GGT is log-linearly associated with increased CVD risk [18], findings which are consistent with several previous studies [19].

Whether a prospective relationship exists between CDT (expressed as %CDT) and risk of CVD is not known. We therefore aimed to evaluate the associations of self-reported alcohol consumption and %CDT with the risk of CVD using the PREVEND study.

2. Methods

2.1. Study design and population

This study was conducted using STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) guidelines for reporting observational studies in epidemiology (Supplementary Material 1) [20]. The participants in this analysis were part of the PREVEND general population-based prospective cohort study, which was designed to evaluate the natural course of urinary albumin excretion and its relationship to renal disease and CVD. Several previous reports have provided detailed description of the study design and recruitment methods [18,21–23]. Briefly, participants in the PREVEND study comprised of a representative sample of inhabitants living in the city of Groningen in the Netherlands. The cohort for this study comprised of 6,894 individuals aged 28–75 years who were invited for the second screening phase of the study, for which baseline assessments were performed between 2001 and 2003. We excluded participants with a history of CVD at baseline. The analytic sample is based on 5,206 participants with complete information on self-reported alcohol consumption, CDT and incident cardiovascular outcomes. The derivation of the analytic sample is reported in Supplementary Material 2. The PREVEND study was approved by the Medical Ethics Committee of the University Medical Center Groningen (#: MEC 96/01/022) and it was conducted in accordance with the Declaration of Helsinki. Written informed consent was provided by all participants.

2.2. Assessment of exposures and other risk markers

Baseline data on sociodemographics, physical measures, medical history and medication use and circulating blood biomarkers were assessed during two outpatient visits by study participants. Following an overnight fast and 15 min of rest, plasma and serum venous samples were taken from participants for biochemical measurements. Plasma samples were prepared by centrifugation at 4 °C. Plasma and serum samples were stored at −80 °C until measurements were done. Total cholesterol, high-density lipoprotein cholesterol (HDL-C), high sensitivity C-reactive protein (hsCRP) and triglycerides were measured using standard laboratory protocols [24–28]. Serum creatinine was determined by Kodak Ektachem dry chemistry (Eastman Kodak, Rochester, New York) and serum cystatin C level by nephelometry (BN II N) (Dade Behring Diagnostic, Marburg, Germany). In 2001–2003 (which was the period of baseline measurements), creatinine assays were non-IDMS calibrated. To allow for the calculation of estimated glomerular filtration rate (eGFR) based on the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) combined creatinine-cystatin C equation, we performed re-measurements of approximately 28,000 available plasma samples of the five PREVEND screenings in 2010–2012 using an IDMS traceable enzymatic Roche assay. Estimated glomerular filtration rate was calculated using the CKD-EPI combined creatinine-cystatin C equation [29]. Plasma glucose was measured by dry chemistry (Eastman Kodak, Rochester, New York). Circulating levels of ferritin, transferrin and CDT concentrations were measured in serum. For these measurements, sampling was performed at the screening phase in 2001–2003. Samples were then aliquoted and stored frozen at −80 °C. These aliquots were retrieved from frozen storage for batchwise analyses. Serum ferritin was measured using an electrochemiluminescence immunoassay (Roche Cobas Diagnostics GmbH, Mannheim Germany). Transferrin was analyzed by immunoturbidimetric assay on a Cobas analyzer (Roche Diagnostics GmbH, Mannheim Germany). The transferrin assay was standardized against the reference preparation of the Institute for Reference Materials and Measurements BCR470/CRM470. The intra-assay and inter-assay coefficients of variation were 1.4 to 1.9 at a level of 1.8 g/L and 1.8% to 1.8% at a level of 2.8 g/L. The detection limit of the assay was 0.1 g/L. Absolute CDT was analyzed and measured in mg/L on a BNII nephelometer (Siemens Healthcare GmbH, Marburg, Germany) [30]. Our approach using the BNII nephelometer did allow for automatic calculation of CDT value as a percentage of total transferrin, because transferrin measurements had already been made on the Roche Modular system. Reference values for absolute CDT ranged from 28.1 to 76.0 mg/L. Its intra-assay and inter-assay coefficients of variation were 2.8% to 4.9% and 1.5% to 7.6%, respectively, depending on the level measured. The detection limit for absolute CDT was 20 mg/L. The %CDT was calculated as the percentage of total transferrin concentrations. The reference values for %CDT ranged from 1.19% to 2.47% (1st-99th percentile) [31]. In comparison, values for %CDT have been reported to range from 1.01 to 2.85% in healthy subjects with an upper reference limit of 2.35% (97.5th percentile) using the N latex CDT direct

immunonephelometric assay for serum CDT [30]. Blood pressure values were recorded as the mean of the last two readings of both visits. Alcohol consumption was obtained by self-report. Participants were asked about the frequency of their habitual alcohol consumption with the following answer categories: (i) no, almost never; (ii) 1–4 units/mo, (iii) 2–7 units/wk, (iv) 1–3 units/d, or (v) > 3 units/d. Based on these 5 categories, alcohol consumption was defined as: abstention, light, light–moderate, moderate, and heavy alcohol consumption.

2.3. Ascertainment of outcomes

First-onset composite CVD was the primary outcome, with incident CHD and stroke as secondary outcomes. All CVD cases were coded according to the *International Classification of Diseases*, Ninth Revision (ICD-9) until 01–01-2009. The outcomes were coded according to ICD-10 codes after this date. Information on hospitalization for cardiovascular morbidity was retrieved from PRISMANT, the Dutch national registry of hospital discharge diagnoses [32]. First-onset CVD was defined as acute myocardial infarction (MI), the combined endpoint of acute and subacute ischemic heart disease (IHD), coronary artery bypass grafting (CABG) or percutaneous transluminal coronary angioplasty (PTCA), subarachnoid hemorrhage, intracerebral hemorrhage, other intracranial hemorrhage, occlusion or stenosis of the precerebral or cerebral arteries and other vascular interventions such as percutaneous transluminal angioplasty or bypass grafting of peripheral vessels and aorta. Coronary heart disease was defined as fatal or nonfatal IHD, fatal or nonfatal MI, CABG and PTCA. Stroke was defined as subarachnoid hemorrhage, intracerebral hemorrhage, other and unspecified intracranial hemorrhage, occlusion and stenosis of precerebral or cerebral arteries and carotid obstruction.

2.4. Statistical analyses

Skewed variables (e.g., triglycerides, hsCRP and creatinine) were natural logarithm ($\log_e$) transformed to achieve normality. Descriptive statistics were used to summarize baseline characteristics of participants. Normally distributed and skewed variables are presented as means (standard deviation, SD) and median (interquartile range, IQR), respectively. Cross-sectional associations of %CDT with risk markers for CVD were assessed using linear regression models adjusted for age and sex. Time-to-event Cox proportional hazards models were used to assess the associations of self-reported alcohol consumption and %CDT with the risk of cardiovascular outcomes, after confirmation of no major departure from the proportionality of hazards assumptions [33]. Hazard ratios were adjusted for in four progressive models: (Model 1) age and sex; (Model 2) plus other established CVD risk factors (smoking status, history of diabetes, SBP, total cholesterol and HDL-C); (Model 3) plus other potential confounders (triglycerides, body mass index (BMI), fasting glucose and eGFR) and (Model 4) plus hsCRP. To minimize risk of bias due to reverse causation, we performed sensitivity analyses that excluded the first two years of follow-up or participants on cholesterol lowering medication. All statistical analyses were conducted using Stata

Table 1
Baseline participant characteristics overall and according to self-reported alcohol consumption.

	Overall (N = 5,206) Mean (SD) or median (IQR) or n (%)	No, almost never (N = 1,278) Mean (SD) median (IQR) or n (%)	1–4 units/mth (N = 889) Mean (SD) or median (IQR) or n (%)	2–7 units/wk (N = 1,655) Mean (SD) or median (IQR) or n (%)	1–3 units/day (N = 1,158) Mean (SD) or median (IQR) or n (%)	>3 units/day (N = 226) Mean (SD) or median (IQR) or n (%)	*P*-value for heterogeneity
%CDT	1.59 (0.54)	1.47 (0.36)	1.46 (0.35)	1.58 (0.50)	1.73 (0.59)	2.22 (1.05)	<0.001
Questionnaire							
Male	2,485 (47.7)	428 (33.5)	368 (41.4)	858 (51.8)	657 (56.7)	52 (23.0)	<0.001
Age at survey (years)	53.0 (11.8)	55.1 (12.6)	53.3 (12.7)	50.8 (11.3)	53.3 (10.8)	54.1 (10.0)	<0.001
History of diabetes	281 (5.4)	113 (8.8)	41 (4.6)	65 (3.9)	54 (4.7)	8 (3.5)	<0.001
Current smoking	1,440 (27.7)	334 (26.1)	207 (23.3)	439 (26.5)	345 (29.8)	115 (50.9)	<0.001
Regular use of anti-hypertensive medication	767 (15.7)	265 (21.7)	128 (15.1)	186 (12.2)	151 (13.8)	37 (17.3)	<0.001
Regular use of lipid-lowering medication	119 (2.8)	38 (3.5)	17 (3)	31 (2.4)	33 (3.5)	0 (0)	0.04
Physical measurements							
BMI (kg/m^2)	26.5 (4.3)	27.5 (5.0)	26.8 (4.4)	26.1 (3.9)	26.0 (3.6)	26.5 (4.2)	<0.001
SBP (mmHg)	126 (18)	127 (20)	124 (18)	124 (17)	127 (18)	131 (17)	<0.001
DBP (mmHg)	73 (9)	73 (9)	72 (9)	73 (9)	74 (9)	78 (8)	<0.001
Lipid markers							
Total cholesterol (mmol/l)	5.47 (1.04)	5.43 (1.07)	5.40 (1.05)	5.45 (1.02)	5.52 (1.01)	5.79 (1.13)	<0.001
HDL-C (mmol/l)	1.27 (0.32)	1.21 (0.29)	1.24 (0.28)	1.28 (0.32)	1.32 (0.33)	1.33 (0.37)	<0.001
Triglycerides (mmol/l)	1.11 (0.80–1.60)	1.17 (0.84–1.67)	1.11 (0.80–1.58)	1.07 (0.77–1.53)	1.09 (0.80–1.59)	1.22 (0.85–1.98)	0.48
Metabolic, inflammatory, and renal function markers							
Transferrin (mg/L)	2582 (405)	2605 (436)	2579 (407)	2577 (402)	2567 (383)	2568 (349)	0.21
hsCRP (mg/L)	1.32 (0.61–2.96)	1.67 (0.76–3.58)	1.34 (0.62–3.02)	1.15 (0.55–2.68)	1.14 (0.55–2.53)	1.64 (0.77–3.56)	<0.001
Fasting plasma glucose (mmol/l)	5.00 (1.10)	5.10 (1.28)	4.90 (0.86)	4.96 (1.11)	5.01 (1.09)	5.05 (0.77)	<0.001
Creatinine (μmol/l)	71 (62–80)	69 (60–78)	69 (61–79)	71 (63–80)	72 (63–81)	74 (64–81)	<0.001
Cystatin C (mg/L)	8.98 (1.95)	9.32 (2.03)	9.00 (1.88)	8.81 (1.63)	8.84 (2.29)	9.00 (1.67)	<0.001
eGFR (ml/min/1.73 m^2)	84.6 (9.8)	82.3 (11.6)	84.4 (9.9)	85.7 (8.4)	85.5 (9.4)	86.1 (8.3)	<0.001

Continuous variables are reported as mean ± SD or median (interquartile range) and categorical variables are reported as n (%); BMI, body mass index; CDT, carbohydrate deficient transferrin; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate (as calculated using the Chronic Kidney Disease Epidemiology Collaboration combined creatinine-cystatin C equation); HDL-C, high-density lipoprotein cholesterol; hsCRP, high sensitivity C-reactive protein; IQR, interquartile range; SBP, systolic blood pressure; SD, standard deviation.

MP version 16 (Stata Corp, College Station, Texas).

3. Results

3.1. Baseline characteristics

Baseline descriptive characteristics of the participants overall and by categories of self-reported alcohol consumption are shown in Table 1. The mean age of participants at study entry was 53 (SD 12) years and 47.7% were males. Mean (SD) of %CDT was 1.59 (0.54) %. Heavy consumers of alcohol had higher levels of %CDT, blood pressure, total cholesterol, hsCRP, fasting glucose and creatinine and were more likely to smoke compared to other categories; whereas abstainers were older, had higher BMI and were more likely to have pre-existing disease such as diabetes and hypertension. When the two alcohol consumption categories "No, almost never" and "1–4 units/mth" were combined, baseline characteristics across categories of self-reported alcohol consumption remained similar except for fasting glucose levels, (Supplementary Material 3).

Percent CDT was weakly and inversely correlated with BMI, triglycerides and transferrin; with weak positive correlations observed with hsCRP and cystatin C (Table 2). A moderately strong positive correlation was observed with HDL-C (r = 0.19). There was a modest positive correlation between self-reported alcohol consumption and % CDT (Spearman's rho = 0.25, $p < 0.001$).

3.2. Self-reported alcohol consumption, %CDT and incident CVD

During a median follow-up of 8.3 (interquartile range, 7.7–8.9) years, corresponding to 40,671 person-years at risk, 326 incident CVD events (annual rate 8.02/1,000 person-years at risk; 95% CI: 7.19–8.93) were recorded. Table 3 shows the associations of alcohol consumption assessed by self-reports and %CDT with the risk of CVD. Compared with abstainers, the HRs (95% CIs) of CVD for light, light–moderate, moderate and heavy alcohol consumption were 0.65 (0.46–0.94), 0.82 (0.61–1.10), 0.80 (0.59–1.10) and 0.81 (0.48–1.36), respectively, in an analysis adjusted for established cardiovascular risk factors. The association remained consistent on additional adjustment for triglycerides, BMI, glucose, and eGFR and was not attenuated following further adjustment for $\log_e$ hsCRP. In separate analyses for CHD and stroke endpoints, the associations of self-reported alcohol consumption with CHD were generally similar to that of the composite CVD outcome; however, for stroke, the association was significant for self-reported light-moderate alcohol consumption (Supplemental Materials 4–5). In sensitivity analyses using the composite CVD endpoint, the associations remained similar on exclusion of the first two years of follow-up or people on cholesterol lowering medication (Supplemental Tables 6–7). In additional analysis that combined the two alcohol consumption categories "No, almost never" and "1–4 units/mth", no associations were observed for alcohol consumption and CVD risk (Supplemental Material 8).

No significant associations were observed for %CDT with composite CVD (Table 3) and individual CHD and stroke endpoints (Supplemental Materials 4–5).

Table 2
Cross-sectional correlates of percent carbohydrate deficient transferrin.

	Partial correlation r (95% CI)†	Percentage difference (95% CI) in %CDT‡
%CDT	–	–
Sex		
Female	–	ref
Male	–	0.11% (0.08, 0.14)***
Age at survey (years)	0.00 (−0.02, 0.03)	0.00% (−0.01, 0.02)
Questionnaire		
History of diabetes		
No	–	ref
Yes	–	−0.04% (−0.11, 0.03)
Smoking status		
Never and former smokers	–	ref
Current smokers	–	0.15% (0.12, 0.18)***
Alcohol consumption		
No	–	ref
Yes	–	0.16% (0.12, 0.19)***
Use of anti-hypertensive medication		
No	–	ref
Yes	–	0.05% (0.00, 0.09)*
Use of lipid-lowering medication		
No		ref
Yes		−0.03% (−0.13, 0.07)
Physical measurements		
BMI (kg/m^2)	−0.09 (−0.12, −0.06)***	−0.05% (−0.07, −0.03)***
SBP (mmHg)	0.01 (−0.02, 0.03)	0.00% (−0.01, 0.02)
DBP (mmHg)	0.04 (0.01, 0.06)*	0.02% (0.00, 0.04)*
Lipid markers		
Total cholesterol (mmol/l)	−0.01 (−0.03, 0.02)	−0.00% (−0.02, 0.01)
HDL-C (mmol/l)	0.19 (0.17, 0.22)***	0.11% (0.10, 0.13)***
Triglycerides (mmol/l)	−0.03 (−0.06, −0.00)*	−0.02% (−0.03, −0.00)*
Metabolic, inflammatory, and renal function markers		
Transferrin (mg/L)	−0.14 (−0.16, −0.11)***	−0.07% (−0.09, −0.06)***
hsCRP (mg/L)	0.04 (0.01, 0.07)*	0.02% (0.01, 0.04)*
Fasting plasma glucose (mmol/l)	−0.02 (−0.05, 0.01)	−0.01% (−0.03, 0.01)
Creatinine (μmol/l)	−0.02 (−0.05, 0.01)	−0.01% (−0.03, 0.01)
Cystatin C (mg/L)	0.05 (0.02, 0.08)**	0.03% (0.01, 0.05)**
eGFR (ml/min/1.73 m^2)	0.01 (−0.02, 0.03)	0.00% (−0.01, 0.02)

BMI, body mass index; CDT, carbohydrate deficient transferrin; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate (as calculated using the Chronic Kidney Disease Epidemiology Collaboration combined creatinine-cystatin C equation); HDL-C, high-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; Ref, reference; SD, standard deviation; SBP, systolic blood pressure.
Asterisks indicate the level of statistical significance: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; †Partial correlation coefficients between %CDT and the row variables; ‡Percentage change in %CDT per 1 SD increase in the row variable (or for categorical variables, the percentage difference in mean %CDT for the category versus the reference) adjusted for age and sex.

4. Discussion

4.1. Summary of main findings

In this large general population-based prospective study, we sought to evaluate the associations of self-reported alcohol consumption and CDT (expressed as %CDT) with the risk of CVD. Correlational analyses demonstrated mostly weak to moderately strong correlations between % CDT and several cardiovascular risk markers. A significant and moderately strong positive correlation was observed between self-reported alcohol consumption and %CDT. Categories of increasing alcohol consumption were continually associated with %CDT values. We confirmed previous consistently reported associations of self-reported alcohol consumption with cardiovascular risk; light to moderate alcohol consumption was associated with decreased cardiovascular risk. However, we could not confirm the association between heavy alcohol consumption and increased risk of CVD [34]. Notably, the association of %CDT with CVD risk was not significant. Findings were robust in sensitivity analyses.

4.2. Comparison with previous work

We are unable to directly compare the current findings with previous work, as our search of the literature did not identify any prospective

Table 3
Prospective associations of self-reported alcohol consumption and %CDT with risk of cardiovascular disease.

Exposure	Events/Total	Model 1 HR (95% CI)	*P*-value	Model 2 HR (95% CI)	*P*-value	Model 3 HR (95% CI)	*P*-value	Model 4 HR (95% CI)	*P*-value
Self-reported alcohol consumption									
No, almost never	102 / 1,278	ref		ref		ref		ref	
1–4 units/mth	42 / 889	0.58 (0.40 to 0.83)	0.003	0.65 (0.46 to 0.94)	0.02	0.66 (0.46 to 0.95)	0.03	0.66 (0.46 to 0.95)	0.02
2–7 units/wk	91 / 1,655	0.77 (0.57 to 1.02)	0.07	0.82 (0.61 to 1.10)	0.19	0.84 (0.62 to 1.12)	0.23	0.83 (0.62 to 1.11)	0.21
1–3 units/d	73 / 1,158	0.74 (0.54 to 1.00)	0.05	0.80 (0.59 to 1.10)	0.17	0.83 (0.60 to 1.14)	0.25	0.83 (0.61 to 1.14)	0.25
>3 units/d	18 / 226	0.79 (0.48 to 1.32)	0.37	0.81 (0.48 to 1.36)	0.43	0.84 (0.50 to 1.41)	0.51	0.80 (0.48 to 1.36)	0.41
Percent carbohydrate deficient transferrin									
Per 1 SD increase	303 / 4,953	1.02 (0.74 to 1.40)	0.92	1.10 (0.79 to 1.54)	0.58	1.10 (0.79 to 1.53)	0.56	1.06 (0.76 to 1.47)	0.75
Quartile 1	80 / 1,520	ref		ref		ref		ref	
Quartile 2	74 / 1,211	1.17 (0.85 to 1.60)	0.34	1.15 (0.84 to 1.58)	0.39	1.17 (0.85 to 1.61)	0.33	1.16 (0.85 to 1.60)	0.35
Quartile 3	87 / 1,280	1.26 (0.93 to 1.71)	0.13	1.22 (0.90 to 1.66)	0.20	1.25 (0.92 to 1.70)	0.15	1.21 (0.89 to 1.65)	0.22
Quartile 4	62 / 942	1.13 (0.81 to 1.57)	0.49	1.20 (0.86 to 1.68)	0.29	1.26 (0.89 to 1.77)	0.19	1.21 (0.86 to 1.71)	0.27

CDT, carbohydrate deficient transferrin; CI, confidence interval; HR, hazard ratio; SD, standard deviation.
Model 1: Age and sex.
Model 2: Model 1 plus smoking status, history of diabetes, systolic blood pressure, total cholesterol, and high-density lipoprotein-cholesterol.
Model 3: Model 2 plus triglycerides, body mass index, glucose, and estimated glomerular filtration rate (as calculated using the Chronic Kidney Disease Epidemiology Collaboration combined creatinine-cystatin C equation).
Model 4: Model 3 plus $\log_e$ high sensitivity C-reactive protein.

study that has evaluated the associations of self-reported alcohol consumption and %CDT with the risk of composite CVD. However, in a longitudinal study conducted as part of the Prospective Urban and Rural Epidemiology (PURE) study in South Africa, self-reported alcohol consumption was only associated with an increased risk of hypertension, but not all-cause mortality or CVD mortality, with no evidence of an association of %CDT with any of the outcomes [35]. In a cross-sectional evaluation of CDT, GGT and self-reported drinking with CHD, Jousilahti and colleagues demonstrated that CDT levels were inversely associated with CHD risk [17]. In the same study, self-reported alcohol consumption was inversely associated with CHD risk in age-adjusted analysis, but this was attenuated to null on further adjustment for several established risk factors including smoking status [17], which has been shown to coincide with heavy alcohol consumption [36].

4.3. Possible explanations for findings

Our findings add to the extensive evidence base on the significant cardioprotective effects of low to moderate alcohol consumption. Though the harmful effects of heavy alcohol consumption on cardiovascular risk and reduced life expectancy are well documented, we could not demonstrate this to be statistically significant in our study. In a recent combined analysis of individual participant data based on over half a million participants without previous CVD, it was demonstrated that there no clear risk thresholds below which lower alcohol consumption stopped being associated with lower cardiovascular risk [8]. It has been reported that 40–60% of the beneficial effect of low to moderate alcohol consumption on the risk of CVD is mediated through an increase in HDL-C alone, with further benefits through reduced hemostatic factors such as fibrinogen levels and clotting factors [37]. In line with our previous report, an increase in alcohol consumption was associated with higher serum activity of the antioxidative enzyme paraoxonase-1 (PON-1), in close parallel with increases in HDL-C and its major apolipoprotein, apolipoprotein A-I (apoA-I); notably, there was no further increase in PON-1 activity, HDL-C and apoA-I when alcohol consumption was increased from moderate to heavy consumption [38]. In our study, the lack of an association between heavy alcohol consumption and CVD risk could be attributed to reduced power in that category (n = 226, 18 CVD events). Given that %CDT has higher specificity for chronic excessive alcohol consumption than other markers such as GGT, AST or MCV, the lack of evidence is unexpected. A number of reasons may explain this observation. Usually, consumption of 50–60 g of alcohol per day chronically (for at least 2 or 3 weeks) increases CDT levels [10]. This level of alcohol consumption is unlikely to be characteristic of the study participants, evidenced by the low mean % CDT values in the study population (1.59%). The levels of alcohol consumption in the study population are not excessive enough to exert cardiotoxic effects, hence, a lack of an association between %CDT and CVD risk. This is also consistent with the lack of an association between self-reported heavy alcohol use and CVD risk in our study participants. Furthermore, CDT is a relatively short-term biomarker, whose sensitivity is decreased during abstinence [39]; it has a half-life of 14–17 days with values returning to normal 3–4 weeks after abstinence [10]. Though it has been demonstrated that hair samples of other objective markers such as ethyl glucuronide, represent a more long-term measure of alcohol consumption lasting several months [40], this has not been demonstrated for CDT [41]. In addition, though GGT and CDT are markers of excessive alcohol consumption, they may reflect different patterns of alcohol intake such as frequency or quantity on the risk of CVD [42]. There are suggestions that GGT levels reflect intensity of consumption, whereas CDT level is influenced by the frequency of consumption [42]. It has also been suggested that the effects of alcohol consumption on CDT may depend on the specific alcoholic beverage consumed. Whitfield and colleagues demonstrated that the effects of beer consumption on indices of iron stores such as aserum iron, transferrin, and ferritin, were greater than those of wine or spirits [43]. In a small 12-week randomized, diet-controlled crossover trial, a significant decrease of serum CDT concentration was observed after 3 weeks of daily consumption of red wine compared with water consumption; with no effect of beer or spirits [44]. CDT is a specific marker of excessive alcohol consumption, that has wide applications including routine detection of heavy alcohol consumption, treatment and monitoring of alcohol-dependent patients and as a screening tool [10]. Taking the overall evidence together, %CDT values within the normal range are unlikely to be a risk indicator for CVD in the general population. It appears data on %CDT may be insufficient to assess the effects of alcoholic beverage intake on CVD risk.

4.4. Strengths and limitations

This study is novel as it is the first comparative long-term prospective evaluations of the associations of self-reported alcohol consumption and %CDT with the risk of composite CVD as well as specific endpoints of CHD and stroke. Other strengths include the large sample size, exclusion of individuals with a baseline history of CVD thus minimizing reverse causation bias and access to a comprehensive panel of cardiovascular risk markers which enabled adjustment for potential confounding. The findings were also robust to several sensitivity analyses. Several

limitations deserve consideration. Categories of alcohol consumption were based on self-reports, which provide limited information and have been criticised due to the potential for misclassification bias [45]. It is possible that some of the light drinkers were probably light-moderate drinkers while some moderate drinkers were likely to be heavy drinkers, due to the inclination of people to under-report consumption. Furthermore, habitual alcohol consumption was divided into 5 categories, and the time since last alcohol consumption was not recorded. Therefore, only the global distinction between abstinent, light, light–moderate, moderate, and heavy drinkers could be made. We could not evaluate the associations of specific types of alcohol beverages with CVD risk. It is well known that there have been inconsistencies regarding the specific effects of different types of beverages (wine, beer and spirits) on CVD risk, and also whether the possible protective effects of alcoholic beverages are due to their alcoholic content (ethanol) or to their non-alcoholic components (mainly polyphenols) [46]. A number of studies have demonstrated that polyphenols in wine and beer may lower CVD risk independent of ethanol [46]. We acknowledge that our %CDT values may not be precise as IFCC standardized procedures were not used. Our analyses were based on single baseline assessments of alcohol intake and %CDT, which may not accurately reflect participants' true long-term "usual" or "average" exposures throughout the duration of the study, due to the phenomenon of regression dilution bias. Based on findings of studies that accounted for regression dilution bias by using information on repeat assessments of self-reported alcohol intake [37], evaluations that use single assessments of this exposure may systematically underestimate the true risk of disease associated with it. Given the limitations, the current findings need to be interpreted with caution.

5. Conclusion

Our findings in a general population cohort of Caucasian men and women confirm the established associations between self-reported light to moderate alcohol consumption and reduced CVD risk. However, %CDT within the normal reference range may not be a risk indicator for CVD.

Authorship

The authors' responsibilities were as follows – DK, MFE, EGG, MHdeB, ACMK, JEK-R, RPFD and SJLB conceived the study; SKK: analyzed data, performed statistical analysis, wrote the paper, and had primary responsibility for final content; and all authors: interpreted the data analysis, and read and approved the final manuscript.

Ethics

The PREVEND study was approved by the Medical Ethics Committee of the University Medical Center Groningen and it was conducted in accordance with the Declaration of Helsinki. Written informed consent was provided by all participants.

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CRediT authorship contribution statement

Setor K. Kunutsor: Methodology, Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **Daan Kremer:** Conceptualization, Writing - review & editing. **Michele F. Eisenga:** Conceptualization, Writing - review & editing. **Eke G. Gruppen:** Conceptualization, Writing - review & editing. **Martin H. de Borst:** Conceptualization, Writing - review & editing. **Anneke C. Muller Kobold:** Conceptualization, Writing - review & editing. **Jenny E. Kootstra-Ros:** Conceptualization, Writing - review & editing. **Robin P. F. Dullaart:** Conceptualization, Methodology, Writing - review & editing, Supervision. **Stephan J.L. Bakker:** Conceptualization, Methodology, Writing - review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2021.05.024.

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