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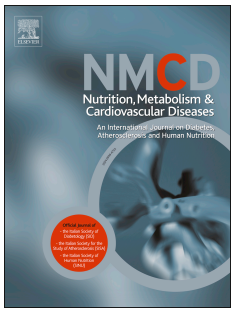

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Serum copper-to-zinc ratio is associated with heart failure and improves risk prediction in middle-aged and older Caucasian men: A prospective study

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Abstract

Background and aims: Serum copper (Cu) and zinc (Zn) may play a role in the development of adverse cardiovascular outcomes including heart failure (HF). Serum Cu/Zn-ratio has been shown to be a risk indicator for cardiovascular disease, but its relationship with HF has not been previously investigated. We aimed to assess the association between Cu/Zn-ratio and incident HF risk using a prospective cohort study

Methods and results: Study participants were recruited in eastern Finland with baseline examinations carried out between March 1998 and December 2001. Serum levels of Cu and Zn were measured using atomic absorption spectrometry in 1,866 men aged 42-61 years without a history of HF at baseline. Multivariable-adjusted hazard ratios (HRs) with confidence intervals (CIs) were calculated for incident HF. During 26.5 years median follow-up**,** 365 HF cases occurred. Restricted cubic splines suggested linear relationships of serum Cu/Zn-ratio, Cu and Zn with HF risk. A unit increase in Cu/Zn-ratio was associated with an increased HF risk in analysis adjusted for several potential confounders including nutritional factors such as total energy intake, intake of fruits, berries and vegetables, and red meat (HR 1.63; 95% CI 1.06-2.51). The corresponding multivariable-adjusted HRs (95% CIs) for serum Cu and Zn were 2.42 (1.32-4.44) and 1.34 (0.50-3.63), respectively. Addition of Cu/Zn-ratio to a HF risk prediction model was associated with improved risk prediction.

Conclusion: In middle-aged and older Finnish men, increased serum Cu/Zn-ratio is associated with an increased risk of HF in a linear dose-response fashion and might improve HF risk assessment.

KEYWORDS: Copper-to-zinc ratio; Copper; Zinc; Heart failure; Risk factor; Cohort study

Introduction

Heart failure is the end-stage manifestation of most forms of cardiovascular disease (CVD). Since heart failure (HF) was designated as a new epidemic in 1997, it persists as a major clinical and public health problem. It has been reported that an estimated 64.3 million people are living with HF globally[1] and it is associated with unacceptably high morbidity and mortality as well as significant economic burden on society.[2] Traditional cardiovascular risk factors such as diabetes, smoking, and hyperlipidaemia play an important role in the pathophysiology of HF, and its incidence and prevalence increase with age.[2] The most common causes of HF include ischemic heart disease, myocardial infarction, hypertension, and valvular heart disease.[3, 4] Due to a wealth of epidemiological studies that have improved our understanding of HF over the last decades and the development of effective strategies to treat and manage the condition, the incidence of HF has stabilized; however, due to the aging of the population, its prevalence is on the increase and the burden of mortality and hospitalization attributable to it remains mostly persistent.[1, 3, 5] These trends reflect the complexity of HF and insufficient understanding of its pathophysiology.[5] Identifying emerging risk factors may hold the key to discovering new mechanistic pathways that lead to HF and the development of new preventive and treatment strategies. For the primary prevention of HF, guidelines of the European Society of Cardiology and the American College of Cardiology, American Heart Association, and Heart Failure Society of America both recommend risk factor modification for those at risk and the use of biomarkers such as for B-type natriuretic peptide (BNP) or N-terminal prohormone of B-type natriuretic peptide (NT-proBNP) for screening.[3] Though markers such as BNP and NT-proBNP are useful for screening, guiding management, and risk prognostication, they are not specific and there is insufficient evidence on their roles.[3, 4] As life expectancy increases, there has been an increasing focus on identifying clinically relevant biomarkers of ageing,[6] which could help identify and prevent aging-related diseases such as HF.

Nutrition is well known to play a major role in the aetiology of chronic diseases. Copper (Cu) and zinc (Zn) are trace elements that are essential for many processes in the human body; they are involved in several cellular processes such as nucleic acid synthesis, enzymatic reactions, oxidoreductases, inflammation, mitochondrial electron transport, cell replication and repair.[7, 8] It

has been reported that circulating Cu and Zn may predict disability and mortality in older people, as they are more related to parameters of inflammation than the nutritional ones.[9, 10] Given their involvement in various biological processes, insufficiency, deficiency, or toxic levels of Cu or Zn can lead to many disease conditions, especially age-related conditions. Both low and high concentrations of Cu have been shown to be associated with an increased risk of CVD.[11-13] A recent meta-analysis of 13 studies reported findings that suggested an association between high serum Cu concentrations and increased HF risk;[14] however, the studies were based on case-control studies which lack temporality. Zinc impacts the cardiovascular system through modulation of oxidative stress and higher serum levels have been demonstrated to be associated with lower risk of CVD.[15] A meta-analysis also based on case-control studies suggested a significant association between low serum Zn levels and increased HF risk.[16] Copper and zinc are biologically interrelated and their concentrations are strictly regulated by compensatory mechanisms that act to stabilize them within certain ranges of nutritional intake; their levels are only slightly affected by nutritional changes unless during severe deficiency or use of supplements.[10] A common feature of diseases characterised by inflammation is an increase in serum Cu and a decrease in serum Zn concentrations.[17] Hence, the typical presentation of many age-related chronic diseases is an increase in the Cu-to-Zn ratio (Cu/Zn-ratio).[10] It has been suggested that the serum Cu/Zn-ratio may be a more reliable risk indicator for adverse health outcomes compared to Cu or Zn alone.[10] High serum Cu/Zn-ratio has shown to be associated with an increased risk of adverse cardiometabolic outcomes including type 2 diabetes,[13] CVD[13, 18] and all-cause mortality.[9] The prospective association between serum Cu/Zn-ratio and the specific outcome of HF has not been previously investigated. Given the nature of the overall existing evidence, we hypothesised that serum Cu/Zn-ratio will be associated with the risk of HF. In this context, our primary objective was to assess the nature, magnitude and specificity of the prospective association between serum Cu/Zn-ratio and HF risk, using a population-based prospective cohort of 1,866 middle-aged and older Finnish men without a history of HF at baseline. Two secondary objectives were to (i) assess the individual associations of serum Cu and Zn with incident HF risk and (ii) assess the extent to which serum Cu/Zn-ratio measurements could improve the prediction of HF in a general population using measures of risk discrimination and reclassification.

Methods

Study design and participants

The conduct of this study was based on STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) guidelines for reporting observational studies in epidemiology (**Supplementary Material 1**). The study protocol was approved by the Research Ethics Committee of the University of Kuopio and all participants included in the study provided written informed consent. All study procedures were adherent to the Declaration of Helsinki. Participants employed in this analysis were part of the Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD), a population-based prospective cohort study that was designed to evaluate risk factors for CVD and other related outcomes. Details of the study setting, study design and recruitment methods have been reported in previous articles.[19-22] Briefly, participants recruited into the KIHD cohort included a representative sample of men aged 42, 48, 54 or 60 years living in the city of Kuopio and surrounding rural communities in eastern Finland. Of the 3433 men who were potentially eligible, 3235 were eligible for inclusion into study. Of this number, 553 did not respond to the invitation or declined to provide informed consent and 2682 volunteered to participate. Baseline examinations were performed between March 1984 and December 1989. For this analysis, we excluded those with missing data on the exposures and potential confounders (n=615) and those with a baseline history of HF (n=201). The current analysis included 1,866 men with complete information on serum measurements of Cu and Zn, relevant covariates, and incident HF events (**Supplementary Material 2**).

Measurement of covariates and outcome ascertainment

Information on covariates was obtained by history, physical examinations and blood sample measurements during the baseline visits. The description of blood sample collection and assays for blood biomarkers, physical measurements, assessment of lifestyle characteristics, medical history and dietary intakes have been reported in previous articles.[23-26] Briefly, before blood samples were taken between 8:00 and 10:00 a.m., participants were required to have fasted overnight and abstained from drinking alcohol for at least 3 days and from smoking for at least 12 hours. Measurements of serum Cu and Zn concentrations were made from frozen serum samples stored at -20° C for 1-5 years,

using the PerkinElmer 306 atomic absorption spectrophotometer (Norwalk, Connecticut, USA) with a flame technique and pyrolytically coated graphite tubes with a platform.[27] Serum Zn concentrations were determined in the same batches with Cu. Seronorm (Nycomed, Oslo, Norway) control serum samples were included in all daily batches. The reference standards were dissolved in 5% glycerol and the between-batch coefficient of variation was 4.0%. The cholesterol content of lipoprotein fractions was assayed enzymatically (Boehringer Mannheim, Mannheim, Germany). Fasting plasma glucose (FPG) was measured by the glucose dehydrogenase method (Merck, Darmstadt, Germany) after protein precipitation by trichloroacetic acid. Serum high sensitivity C-reactive protein (hsCRP) was measured using an immunometric assay (Immulite High-Sensitivity CRP assay, DPC).[28] Estimated glomerular filtration rate (GFR) was derived using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[29] Resting blood pressure was measured on the first examination day using a random-zero mercury sphygmomanometer. After a supine rest of 5 minutes, three measurements in supine, one in standing, and two in sitting positions with 5-minute intervals were taken. The mean of all six systolic blood pressures (SBPs) was used. Body mass index (BMI) was calculated by dividing measured weight in kilograms by the square of height in meters. Self-administered questionnaires were used to assess medical history and lifestyle characteristics such as smoking and alcohol consumption.[23] A participant was defined as a smoker if he had ever smoked regularly and had smoked cigarettes, cigars, or a pipe within the past 30 days.[30] Alcohol consumption was assessed using the Nordic Alcohol Consumption Inventory.[31] A history of CHD was based on a previous myocardial infarction, angina pectoris, the use of nitroglycerin for chest pain once a week or more frequently or chest pain. Data on use of antihypertensive medications were assessed by self-administered questionnaires and from the Social Insurance Institution of Finland register, which contains information on the reimbursement of expenses for medications including antihypertensive ones. A history of diabetes was defined as having a clinical diagnosis of diabetes and regular treatment with diet, oral hypoglycaemic agents or insulin therapy, FPG $\geq$7.0 mmol/l, or according to self-reports. Socioeconomic status (SES) was assessed using self-reported questionnaires via a summary index that combined income, education, occupational prestige, material standard of living and housing conditions. The composite SES index ranged from 0 to 25, with higher values

indicating lower SES.[32] Leisure-time physical activity was assessed from a 12-month physical activity history modified from the Minnesota Leisure-Time Physical Activity Questionnaire.[33] Using household measures, the consumption of foods (including intake of fruits, berries and vegetables, and intake of processed and unprocessed red meat) was assessed with the use of a 4-day guided food record, during three weekdays and one weekend day. A picture book of common foods and dishes was used to help in estimation of portion sizes. The intake of fruits, berries and vegetables also included jams, nectars and juices, but did not include potatoes. Processed red meat included all red meat that had undergone industrial processing, such as the addition of salt or preservatives. Nutrient intakes were estimated with the use of NUTRICA version 2.5 software (Social Insurance Institution, Finland) and these were all energy adjusted with the use of the residual method.[34] Energy adjustment is based on the premise that a larger, more physically active person requires a higher energy intake, which is associated with a higher absolute intake of all nutrients.[35] Instructions were provided and completed food records were checked by a nutritionist together with the participant, to ensure accuracy.

Incident cases of HF that occurred from study entry to 2018 were included in this analysis. In the KIHD study, all participants (using Finnish personal identification codes) are under continuous annual monitoring for the development of new events, including new incident HF.[36] The sources of information on outcomes were based on a comprehensive review of hospital records and discharge diagnoses, inpatient physician claims data, study electrocardiograms (ECGs), and medico-legal reports. The diagnostic classification of HF cases was coded according to the ICD-10 codes (I50.0-I50.9, I11, I42.0-I42.9); diagnosis was based on guidelines of the European Society of Cardiology[37] and which included criteria such as symptoms, signs, laboratory investigations including the determination of natriuretic peptides, chest radiography results, echocardiography as well as electrocardiographic findings.[38-41] Documents were cross-checked in detail by two physicians. The Independent Events Committee, masked to clinical data, performed classification of outcomes.

Statistical analysis

Variables with skewed distributions (e.g., alcohol consumption, physical activity) were natural log

transformed to achieve approximately symmetrical distributions. Descriptive statistics were used to summarise baseline characteristics: means (standard deviation, SD) or median (interquartile range, IQR) for continuous variables and n (percentages) for categorical variables. To assess the cross-sectional associations of serum Cu/Zn-ratio with various risk markers, Pearson's correlation coefficients were estimated using linear regression models adjusted for age. Time-to-event Cox proportional hazards models were used to assess the associations of serum Cu/Zn-ratio, Cu and Zn with risk of HF, after confirmation of no major departure from the proportionality of hazards assumptions using Schoenfeld residuals.[42] Hazard ratios (HRs) with 95% confidence intervals (CIs) for incident HF were adjusted for in three models: (Model 1) age; (Model 2) Model 1 plus BMI, total cholesterol, high-density lipoprotein cholesterol (HDL-C), SBP, smoking status, history of type 2 diabetes (T2D), use of antihypertensives, history of CHD, alcohol consumption, FPG, estimated GFR, total energy intake, intake of fruits, berries and vegetables, and intake of processed and unprocessed red meat; and (Model 3) Model 2 plus SES and leisure-time physical activity. Selection of covariates for adjustment was based on their previously established role as risk factors for HF, evidence from previously published studies, or their potential as confounders based on known associations with the outcome and observed correlations with the exposures using the available data.[43] To explore potential nonlinear dose-response relationships of serum Cu/Zn-ratio, Cu and Zn with incident HF risk, we constructed multivariable restricted cubic splines (RCSs) with knots at the 5^{th}, 35^{th}, 65^{th}, and 95^{th} percentiles of the distribution of the exposures as recommended by Harrell.[44] Serum Cu/Zn-ratio, Cu and Zn were modeled as both continuous (per unit increase) and categorical (tertiles) variables given evidence of linear relationships with HF risk using the RCSs. We constructed Kaplan–Meier curves for tertiles of serum Cu/Zn-ratio and compared them using the log rank test. Formal tests of interaction were used to assess statistical evidence of effect modification by clinically relevant characteristics for the association between serum Cu/Zn-ratio and HF risk; these included age (median cutoff), alcohol consumption (median cutoff), SES (median cutoff), leisure-time physical activity (median cutoff), BMI (obese vs non-obese based on a cutoff of 30 kg/m^2), history of T2D (yes vs no), use of antihypertensives (yes vs no), and history of CHD (yes vs no). To minimize any bias due to reverse causation, sensitivity analysis involved excluding the first two years of follow-up.

To assess whether adding information on serum Cu/Zn-ratio to traditional HF risk factors is associated with improvement in the prediction of HF risk, we calculated measures of discrimination for censored time-to-event data (Harrell's C-index [45]) and reclassification.[46, 47] To investigate the change in C-index on the addition of serum Cu/Zn-ratio, two HF risk prediction models were fitted: one model based on traditional risk factors (i.e., age, BMI, SBP, smoking status, history of T2D, and history of CHD) and the second model with these risk factors plus serum Cu/Zn-ratio**.** The 95% CIs for C-indices and their changes were derived from jackknife standard error. In addition to Harrel's C-index, we tested for differences in the -2 log likelihood of prediction models with and without inclusion of serum Cu/Zn-ratio. The -2 log likelihood test has been recommended as a more sensitive risk discrimination method.[48, 49] Reclassification analyses was restricted to the first 25 years given the long follow-up of the cohort and was assessed using the net-reclassification-improvement (NRI)[46, 47] and integrated-discrimination-improvement (IDI)[46] by comparing the model containing traditional risk factors to the predicted risk from the model containing traditional risk factors plus serum Cu/Zn-ratio. Based on the following parameter specifications: (i) sample size of 1,866 participants; (ii) 19.6% of study participants developing the primary outcome; (iii) level of significance, two-sided test at $\alpha = 0.05$; and (iv) a standard deviation of 0.27 for the exposure, we had 71% power to detect the clinically important HR of 1.63. All statistical analyses were conducted using Stata version MP 17 (Stata Corp, College Station, Texas).

Results

Baseline characteristics

Baseline characteristics of study participants and cross-sectional correlates of serum Cu/Zn-ratio are presented in **Table 1**. The overall mean (standard deviation, SD) age of study participants at baseline was 53 (5) years. The means (SDs) of serum Cu/Zn-ratio, Cu and Zn were 1.21 (0.27), 1.11 (0.18) mg/l and 0.93 (0.12) mg/l, respectively. Significant weak and positive correlations were observed between serum Cu/Zn-ratio and age, alcohol consumption, SES and estimated GFR; whereas, significant weak and inverse correlations were observed with leisure-time physical activity and intake of fruits, berries and vegetables. Serum Cu/Zn-ratio was moderately strongly and positively correlated

with hsCRP (r=0.44). Values of serum Cu/Zn-ratio were significantly higher in men who smoked compared with non-smokers.

Association of serum Cu/Zn-ratio with HF risk

A total of 365 incident cases of HF occurred (annual rate 8.25/1000 person-years at risk; 95% CI 7.44-9.14) during a median (IQR) follow-up of 26.5 (17.8-31.0) years. A multivariable RCS curve showed that the risk of HF increased continuously with increasing serum Cu/Zn-ratio across the range 1.60-3.10 (*p*-value for nonlinearity=.30) (**Figure 1A**). The HR (95% CI) for incident HF per unit increase in serum Cu/Zn-ratio was 1.79 (1.18-2.73) in analysis adjusted for age, BMI, total cholesterol, HDL-C, SBP, smoking status, history of T2D, use of antihypertensives, history of CHD, alcohol consumption, FPG, estimated GFR, total energy intake, intake of fruits, berries and vegetables, and intake of processed and unprocessed red meat, which was minimally attenuated to 1.63 (1.06-2.51) after further adjustment for SES and leisure-time physical activity (**Table 2**). The corresponding adjusted HRs (95% CIs) were 1.23 (0.94-1.60) and 1.16 (0.89-1.51) comparing the top versus bottom tertiles of serum Cu/Zn-ratio. Cumulative hazard curves showed an increased risk of HF among men in the top tertile of serum Cu/Zn-ratio compared with the other Cu/Zn-ratio groups (*p*-value for log-rank test <.001; **Figure 2**). The association between serum Cu/Zn-ratio an HF risk remained consistent across several clinically relevant subgroups except for evidence of interactions by history of T2D and smoking status (*p* for interaction for all=.02). The association between serum Cu/Zn-ratio and HF risk was strong in current smokers but modest in non-smokers (**Figure 3**).

Separate associations of serum Cu and Zn with HF risk

A multivariable RCS curve showed a gradual increase in risk of HF with increasing serum Cu across the range 1.10-2.10 (*p*-value for nonlinearity=.83) (**Figure 1B**). The HR (95% CI) for incident HF per unit increase in serum Cu was 2.71 (1.50-4.89) in analysis adjusted for age, BMI, total cholesterol, HDL-C, SBP, smoking status, history of T2D, use of antihypertensives, history of CHD, alcohol consumption, FPG, estimated GFR, total energy intake, intake of fruits, berries and vegetables, and intake of processed and unprocessed red meat, which was attenuated to 2.42 (1.32-4.44) on additional

adjustment for SES and leisure-time physical activity (**Table 2**). The corresponding adjusted HRs (95% CIs) were 1.51 (1.16-1.96) and 1.41 (1.08-1.84) comparing the top versus bottom tertiles of serum Cu.

A multivariable RCS curve provided weak evidence for a continuous relationship between serum Zn and HF risk (*p*-value for nonlinearity=.19) (**Figure 1C**). There was no evidence of an association between serum Zn and risk of HF when serum zinc was modeled as a continuous or categorical variable (**Table 2**). Given that serum albumin is the major serum carrier of Zn and the documented evidence of an association between hypoalbuminemia and HF risk,[50] we evaluated the association between serum Zn/albumin-ratio and HF risk. There was no significant evidence of an association between serum Zn/albumin-ratio and HF risk (**Supplementary Material 3**). The associations of serum Cu/Zn-ratio, Cu and Zn with risk of HF remained similar in analyses that excluded the first two years of follow-up (**Supplementary Material 4**).

Serum Cu/Zn-ratio and HF risk prediction

A HF risk prediction model containing traditional risk factors yielded a C-index of 0.7301 (95% CI: 0.7077 to 0.7526). After addition of information on serum Cu/Zn-ratio, the C-index was 0.7337 (95% CI: 0.7113 to 0.7562), representing a non-significant increase of 0.0036 (95% CI: -0.0016 to 0.0089; *p*=.18). The -2 log likelihood was significantly improved on addition of serum Cu/Zn-ratio to the risk model (*p* for comparison<.001). The NRI and IDI were 12.99% (95% CI: -35.20 to 61.18; *p*=.60) and 0.0060 (0.0016 to 0.0105; *p*=.008).

Discussion

Key findings

In this prospective evaluation of the association between serum Cu/Zn-ratio and risk of incident HF in middle-aged and older Finnish men without a history of HF, baseline serum Cu/Zn-ratio was weakly correlated with several lifestyle and dietary factors. A modestly strong and significant correlation was observed with hsCRP, reflecting the fact that serum Cu/Zn-ratio is a putative inflammatory marker.

Given that patients with chronic kidney disease have lower serum Zn concentrations[51] and Cu/Zn-ratio is associated with diabetic kidney disease,[52] the positive correlation observed between Cu/Zn-ratio and estimated GFR was unexpected. However, further exploration of the data showed no evidence of a correlation between serum Zn and estimated GFR, which could be attributed to the narrow range of serum Zn values within the study population (0.50-1.62 mg/l). The lack of a correlation between serum Zn and estimated GFR could be driving the observed positive correlation between Cu/Zn-ratio and estimated GFR, however, further study is needed to replicate and understand these unexpected findings. Association analysis showed that elevated serum Cu/Zn-ratio was associated with an increased risk of incident HF, which was consistent with a linear dose-response relationship and independent of several traditional and emerging risk factors for HF. The association remained similar across several clinically relevant subgroups, except for evidence of effect modification by smoking status; the association was strong in current smokers but modest in non-smokers. In separate evaluations of serum Cu and Zn, high serum Cu was associated with increased incident HF risk in a linear dose-response manner, but there was no significant evidence of an association between serum Zn and incident HF risk. The observed associations persisted when the first two years of follow-up were excluded. With regards to the potential utility of serum Cu/Zn-ratio measurements for HF risk assessment, the addition of information on serum Cu/Zn-ratio to two different risk models containing traditional risk factors for HF was associated with an improvement in the discrimination of HF risk using measures such as IDI and the difference in -2 log likelihood, a more sensitive measure when evaluating the added predictive value of a new measurement.

Comparison with previous studies

The current findings cannot be compared to previous work as it is the first prospective evaluation of the association between serum Cu/Zn-ratio and HF risk and the extent to which serum Cu/Zn-ratio could improve the prediction of HF risk. However, a number of observational cohort studies have reported associations between high serum Cu/Zn-ratio and increased risk of adverse vascular outcomes such as CVD[13, 18] and all-cause mortality.[9] Separate evaluations of the associations of serum Cu and Zn with some cardiovascular endpoints have also been reported.[11, 12, 15] A meta-

analysis of 13 studies indicated that patients with HF had higher serum Cu than control subjects.[14] In another meta-analysis of 12 studies, patients with HF had lower zinc levels than their control subjects.[16] Though the findings of these studies suggested evidence of associations between these exposures and HF risk, they were based on case-control study designs which lack temporality. Based on prospective evaluations, we have shown that high serum Cu/Zn-ratio and Cu concentrations are each associated with future risk of HF. Other large-scale and representative prospective studies are needed to confirm or refute the current findings.

Potential underlying mechanisms

Several mechanistic pathways may underline the observed associations of serum Cu/Zn-ratio and Cu concentrations with the risk of incident HF. In addition to their essential roles in almost every cellular process in the human body,[7, 8] Cu and Zn play important roles in the optimal functioning of the cardiovascular system. The human cardiac muscle requires energy from micronutrients to regenerate proteins and cells, and also to support cyclic contractions.[53] In high amounts, serum Cu can exhibit adverse effects. It has been reported that Cu may be an essential mediator of the development and progression of atherosclerotic CVD,[12, 54, 55] which commonly precedes the development of HF. High serum concentrations of Cu may be involved in the pathophysiology of atherosclerotic CVD via (i) oxidative modification of low-density lipoprotein (LDL) cholesterol and free radical formation, which promote atherogenesis [56]; (ii) insulin resistance and pathogenesis of diabetes [57], a major risk factor for CHD; and (iii) luminal narrowing of the arteries, due to expansion of the arterial neointima caused by extracellular matrix molecules, whose major component is Cu.[58] Furthermore, the association between high serum Cu concentrations and increased HF risk may reflect increased concentrations of ceruloplasmin, an acute phase reactant which is the main transport protein for serum Cu (carries 95% of circulating Cu).[59, 60] Ceruloplasmin may promote the development of atherosclerosis via inflammation processes and the formation of reactive oxygen species and LDL-oxidation.[60] The strong association between serum Cu/Zn-ratio and HF risk in current smokers is likely attributed to the chronic exposure to cadmium, which leads to reduced renal reabsorption of Zn subsequently causing decreased serum Zn concentrations and increased serum Cu/Zn-ratio.[61]

Though we were unable to demonstrate a significant evidence of an association between serum Zn and HF risk, several potential pathophysiological pathways that might link serum Zn deficiency to increased risk of HF include increased systemic inflammation due to reduction in antioxidant enzyme activities, increased oxidative stress, increased autophagy and hypertrophy of the myocardium, apoptosis and myocardial necrosis, and degeneration of cardiomyocytes.[62, 63] Given that an increased serum Cu/Zn-ratio is a typical presentation in older people due to their comorbidities, there is also a possibility that the findings of an increased HF risk with an increased serum Cu/Zn-ratio could be due to reverse causation. However, this is unlikely given that men with HF at baseline were excluded and the findings were essentially similar on excluding the first two years of follow-up.

Implications of findings

The overall evidence suggests that serum Cu/Zn-ratio and Cu concentrations could be risk indicators for incident HF. Though the current findings cannot confirm causality due to the observational study design, findings may have several implications for the development of HF prevention strategies. There is emerging evidence that serum Cu/Zn-ratio may be a valuable prognostic marker for age-related chronic conditions.[10] Indeed, the current findings suggest that measurement of the serum Cu/Zn-ratio could potentially be used to identify individuals at high risk of HF on top of established risk factors such as age, BMI, SBP, smoking status, history of T2D, and history of CHD. Measurements of these trace elements are inexpensive, do not require a lot of resources and could be measured as part of the routine blood screening panel. Given that this is the first-ever evaluation on the topic, further larger-scale studies are warranted to confirm the relationship between serum Cu/Zn-ratio and HF risk and evaluate the potential predictive value of serum Cu/Zn-ratio beyond traditional risk factors. A high serum Cu/Zn-ratio is usually caused by increased Cu concentrations and decreased Zn concentrations. Since Zn deficiency in old age is commonly due to insufficient dietary Zn consumption, reduced intestinal absorption or increased losses,[64] its supplementation could correct the deficiency and provide the optimal serum Cu/Zn-ratio necessary to prevent disease. A growing body of evidence suggests a potential therapeutic role of zinc supplementation in the

management of HF.[62] For example, Zn supplementation in HF patients has been shown to improve left ventricular ejection fraction.[65]

Strengths and limitations

We have conducted the first prospective evaluation of the association between serum Cu/Zn-ratio and the specific outcome of HF in addition to assessing the individual associations of serum Cu and Zn with incident HF risk. The sample was relatively large and representative of middle-aged to older Finnish males without a history of HF at baseline. The follow-up period was sufficiently long to ascertain the risk for HF events in the general population. The analyses were comprehensive and included adjustment for several relevant potential confounders, assessment of the dose-response relationships, evaluation for effect modification using several clinically relevant characteristics and risk prediction analysis. We carried out additional analysis excluding the first two years of follow-up to minimise reverse causation bias. The limitations which were mostly inherent to the study design included (i) the inability to generalise findings to other ethnicities, age groups and women; it has been reported that there are gender variations in levels of Cu and Zn;[66] (ii) the possibility that serum Cu concentrations may not accurately reflect actual Cu status, given that leucocyte Cu measurement is regarded as a more reliable index of Cu status in the body;[11] (iii) the stability of serum samples of Cu and Zn could have been affected given the prolonged storage of serum samples (1–5 years); however, Cu and Zn concentrations have been shown not to be affected by prolonged storage in frozen serum samples (at -20°C) for several years or repeated freeze–thaw cycles;[67, 68] (iv) inability to assess the differential impact of the exposures on the risk of HF with preserved versus reduced ejection fractions because there were no detailed echocardiographic data on ventricular systolic and diastolic function; (v) the potential for regression dilution bias given the use of single baseline measurements of the exposures and potential for lifestyle changes, incident diseases and use of medications during the follow-up period; (vi) other biases in observational cohort studies such as residual confounding; and (vii) our analyses were slightly underpowered, hence, the imprecise estimates observed.

In conclusion, increased serum Cu/Zn-ratio is associated with an increased risk of HF in a

linear dose-response fashion and might improve HF risk assessment in middle-aged and older Finnish men. Increased serum Cu concentrations are also associated with increased HF risk in a linear dose-response fashion, but there is no significant evidence of an association between serum Zn and HF risk.

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Authors contribution

SKK: Conceptualization, data analysis, statistics & writing manuscript. AV: Conceptualization & providing valid criticism. SK: Conceptualization & providing valid criticism. JAL: Conceptualization & providing valid criticism. All the authors discussed the data and approved the final version of the manuscript.

Conflict of interest

None

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Figure legends

Figure 1. Restricted cubic splines of the hazard ratios of incident heart failure with serum Cu/Zn-ratio, Cu and Zn

A) Serum Cu/Zn-ratio and HF; B) Serum Cu and HF; C) Serum Zn and HF

Dashed lines represent the 95% confidence intervals for the spline model (solid line).

Models were adjusted for age, body mass index, total cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, smoking status, history of type 2 diabetes, use of antihypertensives, history of coronary heart disease, alcohol consumption, fasting plasma glucose, estimated glomerular filtration rate, total energy intake, intake of fruits, berries and vegetables, and intake of processed and unprocessed red meat; Cu, copper; HF, heart failure; Zn, zinc

Figure 2. Cumulative Kaplan-Meier curves for heart failure during follow-up according to tertiles of serum Cu/Zn-ratio

Cu, copper; Zn, zinc

Figure 3. Association between serum Cu/Zn-ratio and heart failure risk across several clinically relevant subgroups

Hazard ratios were adjusted for age, body mass index, total cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, smoking status, history of type 2 diabetes, use of antihypertensives, history of coronary heart disease, alcohol consumption, fasting plasma glucose, estimated glomerular filtration rate, total energy intake, intake of fruits, berries and vegetables, and intake of processed and unprocessed red meat

CHD, coronary heart disease; CI, confidence interval; Cu, copper; HR, hazard ratio; PA, physical activity; T2D, type 2 diabetes; Zn, zinc

*, *p*-value for interaction; cut-offs used for age, alcohol consumption, socioeconomic status and physical activity are median values; obese was defined as body mass index ≥30 kg/m^2 and non-obese as body mass index <30 kg/m^2

Table 1. Baseline characteristics of study participants and cross-sectional correlates of serum copper-to-zinc ratio

Characteristics	Mean (SD) or median (IQR) or n (%)	Pearson correlation r (95% CI)[a]	Percentage difference (95% CI) in values of percentage of Cu/Zn-ratio per 1 SD higher or compared to reference category of correlate[b]
Serum copper-to-zinc ratio	1.21 (0.27)	-	-
Serum copper, mg/l	1.11 (0.18)	-	-
Serum zinc, mg/l	0.93 (0.12)	-	-
Self-reported clinical and sociodemographic parameters			
Age (years)	53 (5)	0.12 (0.07, 0.16)***	0.03% (0.02, 0.04)***
Alcohol consumption, g/week	42.5 (12.7-106.0)	0.17 (0.12, 0.21)***	0.05% (0.03, 0.06)***
Leisure-time physical activity, KJ/day	1212 (672-1988)	-0.06 (-0.11, -0.02)*	-0.02% (-0.03, -0.00)*
Socio-economic status	8.20 (4.23)	0.15 (0.11, 0.20)***	0.04% (0.03, 0.06)***
History of type 2 diabetes			
No	1800 (96.5)	-	ref
Yes	66 (3.5)	-	-0.05% (-0.12, 0.01)
Current smoking			
No	1224 (65.6)	-	ref
Yes	642 (34.4)	-	0.12% (0.09, 0.14)***
Use of antihypertensives			
No	1533 (82.2)		ref
Yes	333 (17.9)		0.02% (-0.02, 0.05)
History of CHD			
No	1471 (78.8)	-	ref
Yes	395 (21.2)	-	0.03% (0.00, 0.06)*
Physical measurements			
BMI, kg/m^2	26.8 (3.5)	-0.04 (-0.08, 0.01)	-0.01% (-0.02, 0.00)
SBP, mmHg	133 (16)	0.02 (-0.02, 0.07)	0.01% (-0.01, 0.02)
DBP, mmHg	89 (10)	0.00 (-0.04, 0.05)	0.00% (-0.01, 0.01)
Blood-based markers			
Total cholesterol, mmol/l	5.93 (1.00)	0.02 (-0.03, 0.06)	0.00% (-0.01, 0.02)
HDL-C, mmol/l	1.31 (0.31)	0.01 (-0.03, 0.06)	0.00% (-0.01, 0.02)
Fasting plasma glucose, mmol/l	5.33 (1.20)	0.02 (-0.03, 0.06)	0.00 (-0.01, 0.02)
Estimated GFR	87.2 (17.3)	0.16 (0.11, 0.20)***	0.04% (0.03, 0.06)***
High sensitivity C-reactive protein, mg/l	1.26 (0.69-2.38)	0.44 (0.40, 0.47)***	0.12% (0.11, 0.13)***
Dietary intakes			
Total energy intake, kJ/day	9845 (2551)	0.00 (-0.04, 0.05)	0.00% (-0.01, 0.01)
Processed and unprocessed red meat, g/day	148 (77)	0.04 (-0.01, 0.08)	0.01% (-0.00, 0.02)
Fruits, berries and vegetables, g/day	248 (151)	-0.13 (-0.18, -0.09)***	-0.04 (-0.05, -0.02)***

BMI, body mass index; CHD, coronary heart disease; DBP, diastolic blood pressure; GFR, glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; SD, standard deviation; SBP, systolic blood pressure

[a], Pearson correlation coefficients between serum Cu/Zn-ratio and the row variables;. [b], Percentage change in values of serum Cu/Zn-ratio per 1-SD increase in the row variable (or for categorical variables, the percentage difference in mean values of serum Cu/Zn-ratio for the category versus the reference); asterisks indicate the level of statistical significance: *, p<0.05; **, p<0.01; ***, p<0.001

Table 2. Associations of serum copper, zinc and copper-to-zinc ratio with risk of heart failure

Exposure	Events/ Total	Model 1		Model 2		Model 3	
		HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Serum copper-to-zinc ratio							
Per unit increase	365 / 1866	2.33 (1.58-3.44)	< 0.001	1.79 (1.18-2.73)	.007	1.63 (1.06-2.51)	.027
T1 (0.48-1.07)	106 / 623	ref		ref		ref	
T2 (1.08-1.27)	131 / 621	1.23 (0.95-1.59)	.12	1.11 (0.85-1.44)	.45	1.07 (0.82-1.39)	.63
T3 (1.28-3.12)	128 / 622	1.48 (1.14-1.91)	.003	1.23 (0.94-1.60)	.13	1.16 (0.89-1.51)	.28
Serum copper, mg/l							
Per unit increase	365 / 1866	5.25 (3.06-9.00)	< .001	2.71 (1.50-4.89)	.001	2.42 (1.32-4.44)	.004
T1 (0.50-1.02)	101 / 653	ref		ref		ref	
T2 (1.03-1.16)	120 / 594	1.38 (1.06-1.80)	.017	1.26 (0.96-1.65)	.094	1.21 (0.93-1.59)	.16
T3 (1.17-2.12)	144 / 619	2.04 (1.58-2.63)	< .001	1.51 (1.16-1.96)	<.001	1.41 (1.08-1.84)	.011
Serum zinc, mg/l							
Per unit increase	365 / 1866	1.72 (0.66-4.48)	.26	2.20 (0.44-3.23)	.72	1.34 (0.50-3.63)	.56
T1 (0.50-0.89)	118 / 625	ref		ref		ref	
T2 (0.90-0.97)	126 / 631	0.93 (0.72-1.19)	.55	0.97 (0.75-1.25)	.81	0.98 (0.76-1.26)	.88
T3 (0.98-1.62)	121 / 610	1.05 (0.82-1.36)	.70	0.98 (0.76-1.28)	.90	1.01 (0.78-1.32)	.92

CI, confidence interval; HR, hazard ratio; ref, reference; T, tertile

Model 1: Adjusted for age

Model 2: Model 1 plus body mass index, total cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, smoking status, history of type 2 diabetes, use of antihypertensives, prevalent coronary heart disease, alcohol consumption, fasting plasma glucose, estimated glomerular filtration rate, total energy intake, intake of fruits, berries and vegetables, and intake of processed and unprocessed red meat

Model 3: Model 2 plus socioeconomic status and leisure-time physical activity

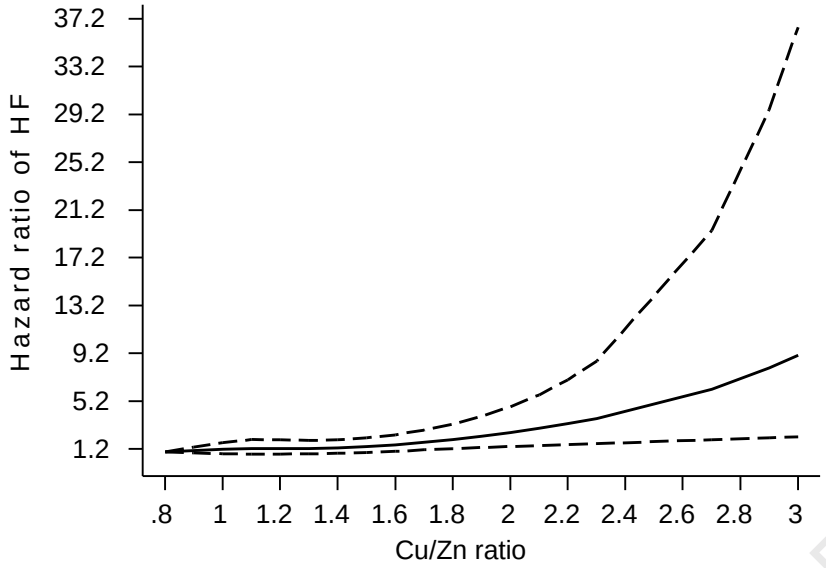
Table 3. Risk discrimination and reclassification upon addition of serum copper-to-zinc ratio to a risk prediction model containing heart failure risk factors

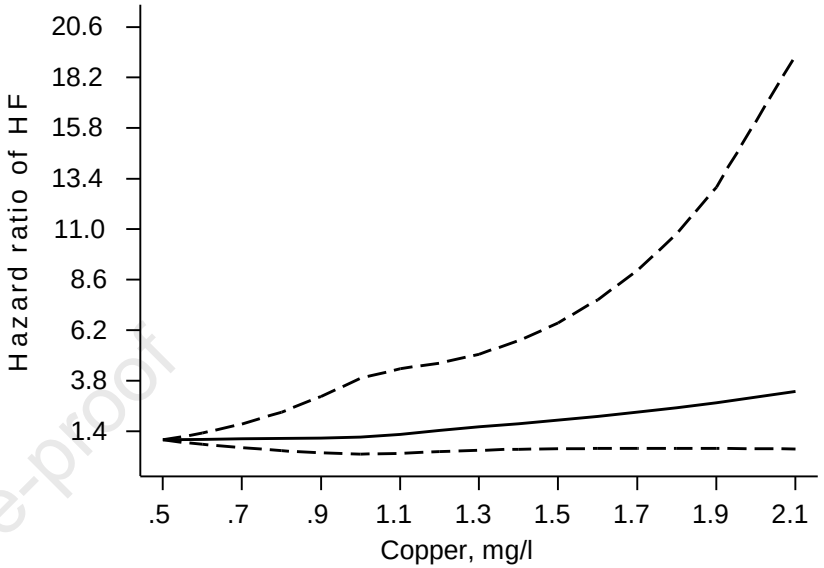
Discrimination	
C-index (95% CI): conventional risk factors	0.7301 (0.7077 to 0.7526)
C-index (95% CI): conventional risk factors plus Cu/Zn-ratio	0.7337 (0.7113 to 0.7562)
C-index change (95% CI)	0.0036 (-0.0016 to 0.0089)
p-value	.18
p-value for difference in -2 log likelihood	<.001
Reclassification	
Continuous Net reclassification index (95% CI)	12.99% (-35.20 to 61.18)
p-value	.60
Integrated discrimination index (95% CI)	0.0060 (0.0016 to 0.0105)
p-value	.008

CI, confidence interval; Cu, copper; Zn, zinc

The model with traditional risk factors included age, body mass index, systolic blood pressure, smoking status, history of type 2 diabetes, and history of coronary heart disease

(A)





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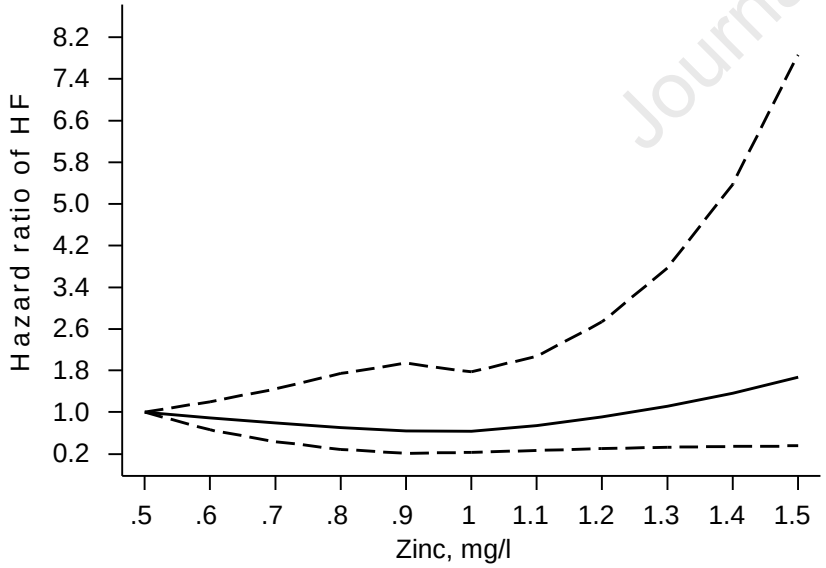
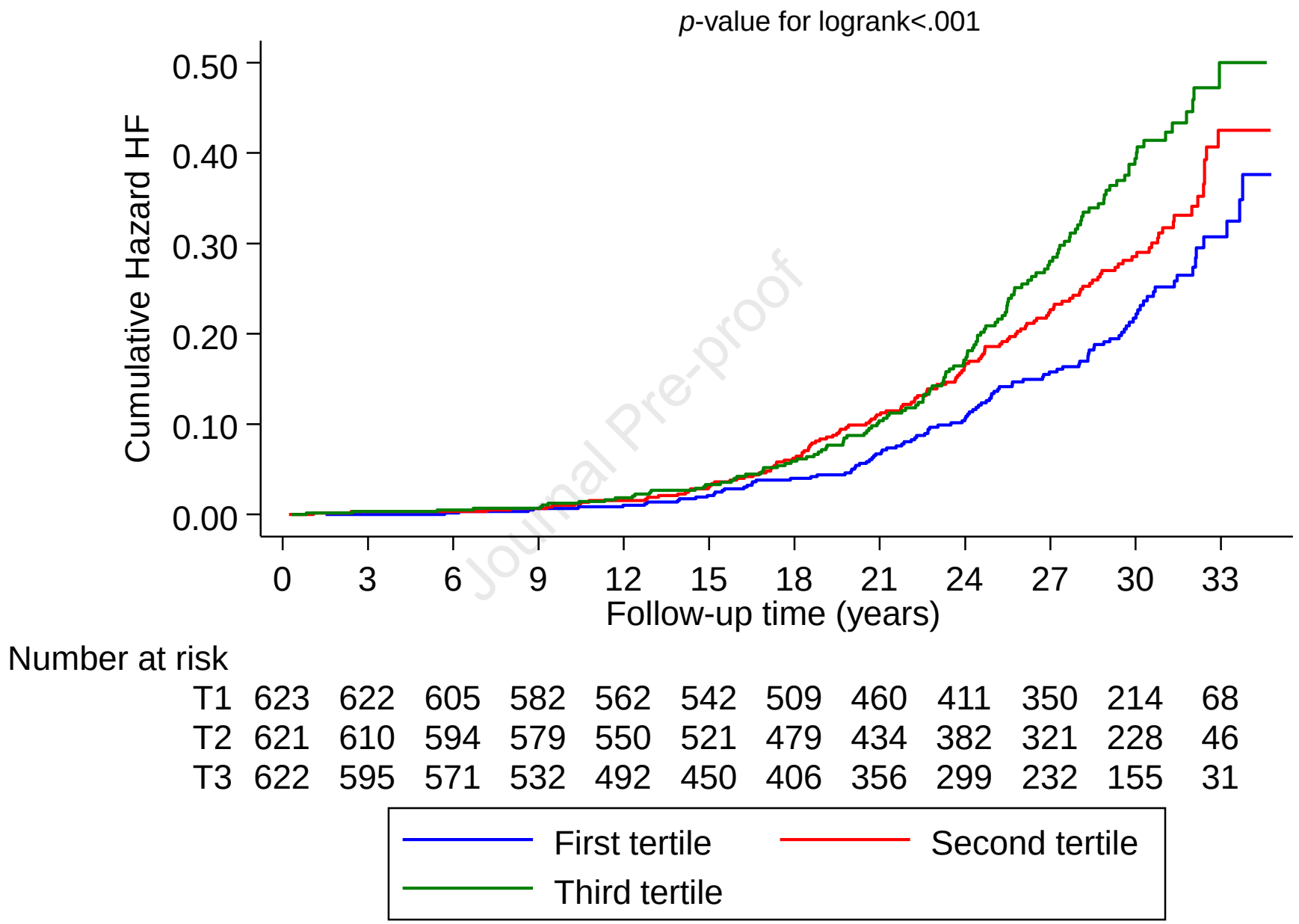
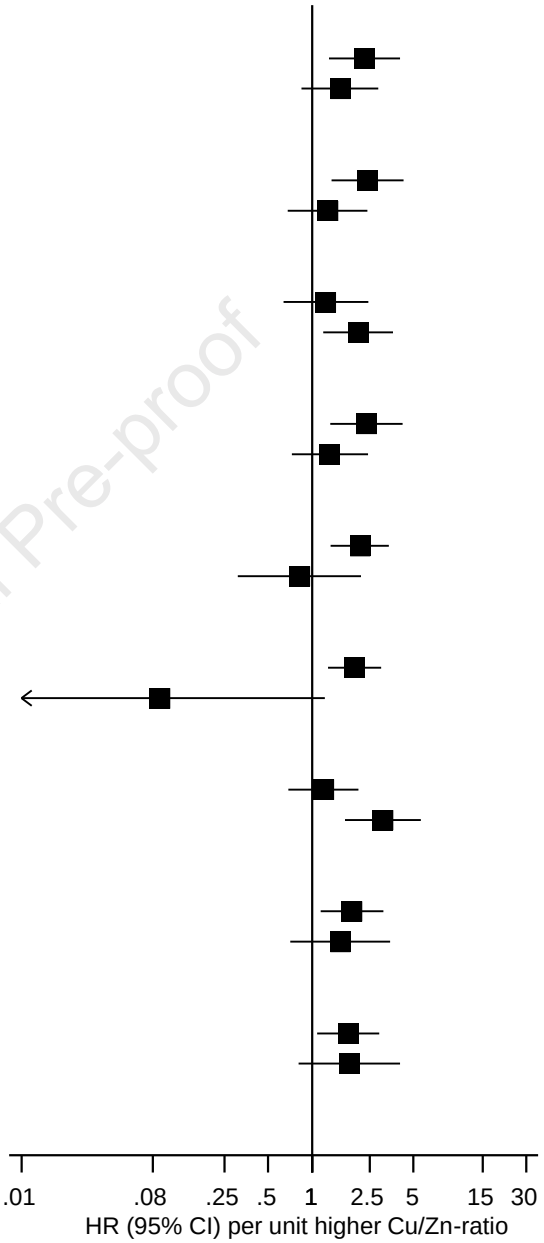




Subgroup	No. of participants	No. of HF cases	HR (95% CI)	*p*-value*
Age at survey (years)				
<55	1,029	167	2.30 (1.31, 4.04)	0.36
≥55	837	198	1.57 (0.85, 2.87)	
Alcohol consumption (g/week)				
<42.5	933	189	2.42 (1.37, 4.29)	0.14
≥42.5	933	176	1.29 (0.68, 2.41)	
Socioeconomic status				
<9	960	151	1.25 (0.64, 2.45)	0.24
≥9	906	214	2.09 (1.20, 3.63)	
Leisure-time PA (KJ/day)				
<1218	933	187	2.38 (1.34, 4.22)	0.17
≥1218	933	178	1.33 (0.73, 2.44)	
Body mass index (kg/m^2)				
Non-obese	1,559	276	2.15 (1.35, 3.40)	0.08
Obese	307	89	0.83 (0.31, 2.18)	
History of T2D				
No	1,800	343	1.97 (1.29, 3.01)	0.02
Yes	66	22	0.09 (0.01, 1.23)	
Smoking status				
Non-smokers	1,224	240	1.21 (0.69, 2.10)	0.02
Current smokers	642	125	3.09 (1.69, 5.65)	
Use of antihypertensives				
No	1,533	248	1.89 (1.15, 3.11)	0.70
Yes	333	117	1.57 (0.71, 3.47)	
History of CHD				
No	1,471	247	1.79 (1.09, 2.92)	0.97
Yes	395	118	1.81 (0.81, 4.04)	



Highlights

- High serum Cu/Zn-ratio is associated with increased HF risk
- High serum Cu is associated with increased HF risk
- Serum Cu/Zn-ratio and Cu are each linearly associated with HF
- Serum Cu/Zn-ratio improves HF risk prediction