Serum Calcification Propensity and CPP2 Size – Deranged Mineral Buffering and The Risk of Mortality in Older Adults

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Supplementary Table 1 ATC Codes of the drug classes defined

Drug group	ATC codes
Vitamin D Supplements	A11CC
Erythropoiesis-stimulating Agents	B03XA
Calcium Supplements	A12A
Vitamin K Antagonists	B01AA
Lipid-lowering Drugs	C10AA05, C10BX12, C10BX06, C10BX19, C10BX11, C10BX18, C10BX08, C10BX03, C10BA05, C10BA08, C10BX15, C10AA01, C10BX04, C10BX01, C10BA02, C10BA04, C10AA07, C10BX07, C10BX14, C10BX13, C10BX05, C10BX09 C10BA06, C10BA09, C10BX16, C10BA07, C10BX17, C10BX20, C10BX10, C10AA04, C10AB02, C10AX15, C10BA10, C10AX14, C10AX13, C10AX16

Supplementary Table 2 Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Abstract
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Introduction
Objectives	3	State specific objectives, including any prespecified hypotheses	Last paragraph Introduction
Methods			
Study design	4	Present key elements of study design early in the paper	Methods – Study population
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Methods – Study population
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	Methods – Study population/ Study design
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	Not applicable
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Methods - Exposures: Mineral stress parameters T_{50} and CPP2, Outcomes (all-cause/cardiovascular mortality) and Covariates
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Methods - Covariates
Bias	9	Describe any efforts to address potential sources of bias	
Study size	10	Explain how the study size was arrived at	Methods – Study population/ Study design
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Methods –Statistical analysis
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Methods –Statistical analysis
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	

		(*d*) If applicable, explain how loss to follow-up was addressed (*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram	Supplementary Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) Summarise follow-up time (eg, average and total amount)	Table 1 and 2
Outcome data	15*	Report numbers of outcome events or summary measures over time	Table 5
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Table 5, Figures 1 and 4
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Figures 2-3 and 5-6
Discussion			
Key results	18	Summarise key results with reference to study objectives	Discussion – 1st paragraph
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Discussion – before last paragraph
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Discussion
Generalisability	21	Discuss the generalisability (external validity) of the study results	Discussion
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Funding paragraph

*Give information separately for exposed and unexposed groups.

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

Supplementary Table 3. Univariate linear regression models for T_{50}.

Variable	Category	T_{50} (min)	
		No. of participants n (%)	Coef. (95% CI)
Age (years)		-	-1.15 (-1.66, -0.64)
Sex	Male	720 (46.0%)	9.51 (2.93, 16.10)
Smoking	Ever	763 (48.7%)	2.30 (-4.28, 8.87)
Physical Activity	<1x/week	495 (31.6%)	Ref.
	1-5x/week	781 (49.9%)	5.65 (-1.82, 13.13)
	>5x/week	283 (18.1%)	2.49 (-7.2, 12.19)
Alcohol Intake	<1x/month	724 (46.2%)	Ref.
	≤2x/week	555 (35.4%)	-1.45 (-8.78, 5.88)
	Regularly	285 (18.2%)	-7.81 (-16.90, 1.28)
Body Mass Index (kg/m^2)	<30	1186 (75.7%)	Ref.
	≥30	373 (23.8%)	6.34 (-1.37, 14.06)
CPP2 size (nm)		-	-0.64 (-0.71, -0.58)
$eGFR_{BIS2}$ ($ml/min/1.73\ m^2$)		-	0.30 (0.08, 0.52)
Biochemical Parameters			
Calcium (mmol/l)		-	59.05 (38.30, 79.81)
$Calcium_{corrected}$ (mmol/l)		-	24.93 (1.28, 48.57)
Albumin (g/l)		-	3.66 (2.67, 4.65)
C-Reactive Protein (mg/l)		-	-1.37 (-1.94, -0.80)
HDL-C (mg/dl)		-	-0.28 (-0.47, 0.10)
LDL-C (mg/dl)		-	0.05 (-0.03, 0.13)
Total Cholesterol (mg/dl)		-	0.03 (-0.04, 0.10)
Triglyceride (mg/dl)		-	0.06 (0.02, 0.09)
Hemoglobin (g/dl)		-	8.88 (6.53, 11.22)
Phosphate (mmol/l)			-85.3 (-99.1, -71.5)
Cardiovascular Parameters			
Systolic BP (mmHg)		-	0.17 (0.01, 0.32)
Diastolic BP (mmHg)		-	0.17 (-0.10, 0.43)
Pulse Pressure (mmHg)		-	0.15 (-0.03, 0.33)

BP: blood pressure; CPP2: calciprotein particle 2 size; eGFR: estimated glomerular filtration rate; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol

Supplementary Table 4. Univariate linear regression models for CPP2 size.

Variable	Category	CPP2 size (nm)	
		No. of participants n (%)	**Coef. (95% CI)**
Age (years)		-	2.18 (1.84, 2.52)
Sex	Male	720 (46.0%)	14.40 (9.86, 19.00)
Smoking	Ever	763 (48.7%)	4.12 (-0.48, 8.73)
Physical Activity	<1x/week	495 (31.6%)	Ref.
	1-5x/week	781 (49.9%)	-9.03 (-14.23, -3.83)
	>5x/week	283 (18.1%)	-4.37 (-11.12, 2.38)
Alcohol Intake	<1x/month	724 (46.2%)	Ref.
	≤2x/week	555 (35.4%)	0.46 (-4.67, 5.58)
	Regularly	285 (18.2%)	10.67 (4.32, 17.02)
Body Mass Index (kg/m^2)	<30	1186 (75.7%)	Ref.
	≥30	373 (23.8%)	-21.19 (-26.50, -15.88)
T_{50} (min)		-	-0.32 (-0.35, -0.29)
$eGFR_{BIS2}$ ($ml/min/1.73\ m^2$)		-	-0.51 (-0.67, -0.36)
Biochemical Parameters			
Calcium (mmol/l)		-	-37.90 (-52.47, -23.32)
$Calcium_{corrected}$ (mmol/l)		-	-12.37 (-28.95, 4.21)
Serum Albumin (g/l)		-	-2.57 (-3.26, -1.87)
C-Reactive Protein (mg/l)		-	1.13 (0.73, 1.53)
HDL-C (mg/dl)		-	0.48 (0.35, 0.61)
LDL-C (mg/dl)		-	-0.36 (-0.42, -0.31)
Total Cholesterol (mg/dl)		-	-0.32 (-0.37, -0.28)
Triglyceride (mg/dl)		-	-0.22 (-0.25, -0.20)
Hemoglobin (g/dl)		-	-7.52 (-9.14, -5.89)
Phosphate (mmol/l)		-	13.9 (3.81, 24.0)
Cardiovascular Parameters			
Systolic BP (mmHg)		-	-0.32 (-0.43, -0.21)
Diastolic BP (mmHg)		-	-0.58 (-0.76, -0.39)
Pulse Pressure (mmHg)		-	-0.17 (-0.29, -0.04)

BP: blood pressure; eGFR: estimated glomerular filtration rate; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; T_{50}: calciprotein crystallization time

Supplementary Table 5 Interaction of age, sex, eGFR, phosphate and corrected calcium on the association of CPP2 size and T_{50} with all-cause and cardiovascular mortality.

		All-cause Mortality				Cardiovascular Mortality			
Marker		**CPP2 size**		**T_{50}**		**CPP2 size**		**T_{50}**	
Model		**Crude**	**Model 1**	**Crude**	**Model 1**	**Crude**	**Model 1**	**Crude**	**Model 1**
Age Models									
70 - <80 years	Number of Events	217 / 703 (31.0%)				75 / 703 (10.7%)			
	HR (95% CI)	1.22 (1.04 – 1.44)	1.18 (1.00 – 1.40)	1.10 (0.97 – 1.25)	1.11 (0.98 – 1.26)	1.33 (1.01 – 1.73)	1.27 (0.97 – 1.67)	1.02 (0.82 – 1.27)	1.04 (0.84 – 1.29)
>80 years	Number of Events	577 / 827 (69.8%)				265 / 827 (32.0%)			
	HR (95% CI)	1.31 (1.21 – 1.41)	1.18 (1.09 – 1.29)	1.10 (1.02 – 1.19)	1.10 (1.02 – 1.18)	1.30 (1.16 – 1.45)	1.15 (1.02 – 1.31)	1.03 (0.92 – 1.15)	1.01 (0.91 – 1.13)
Sex Models*									
Male	Number of Events	433 / 708 (61.2%)				186 / 708 (26.3%)			
	HR (95% CI)	1.45 (1.33 – 1.57)	1.25 (1.14 – 1.37)	1.21 (1.11 – 1.32)	1.13 (1.04 – 1.24)	1.42 (1.25 – 1.62)	1.20 (1.04 – 1.38)	1.25 (1.09 – 1.43)	1.17 (1.02 – 1.33)
Female	Number of Events	361 / 822 (43.9%)				154 / 822 (18.7%)			
	HR (95% CI)	1.31 (1.17 – 1.46)	1.07 (0.95 – 1.22)	1.13 (1.02 – 1.25)	1.06 (0.96 – 1.17)	1.41 (1.21 – 1.65)	1.13 (0.93 – 1.36)	0.92 (0.79 – 1.07)	0.88 (0.76 – 1.01)
eGFR Models									
eGFR ≤56.2	Number of Events	514 / 768 (66.9%)				239 / 768 (31.1%)			
	HR (95% CI)	1.40 (1.29 – 1.52)	1.19 (1.09 – 1.30)	1.10 (1.02 – 1.19)	1.08 (1.00 – 1.17)	1.42 (1.26 – 1.59)	1.21 (1.06 – 1.38)	1.01 (0.90 – 1.13)	0.99 (0.88 – 1.11)
eGFR >56.2	Number of Events	280 / 762 (36.8%)				101 / 762 (13.3%)			
	HR (95% CI)	1.33 (1.17 – 1.50)	1.16 (1.02 – 1.33)	1.18 (1.06 – 1.33)	1.15 (1.03 – 1.30)	1.34 (1.10 – 1.64)	1.09 (0.87 – 1.37)	1.18 (0.97 – 1.42)	1.13 (0.93 – 1.37)

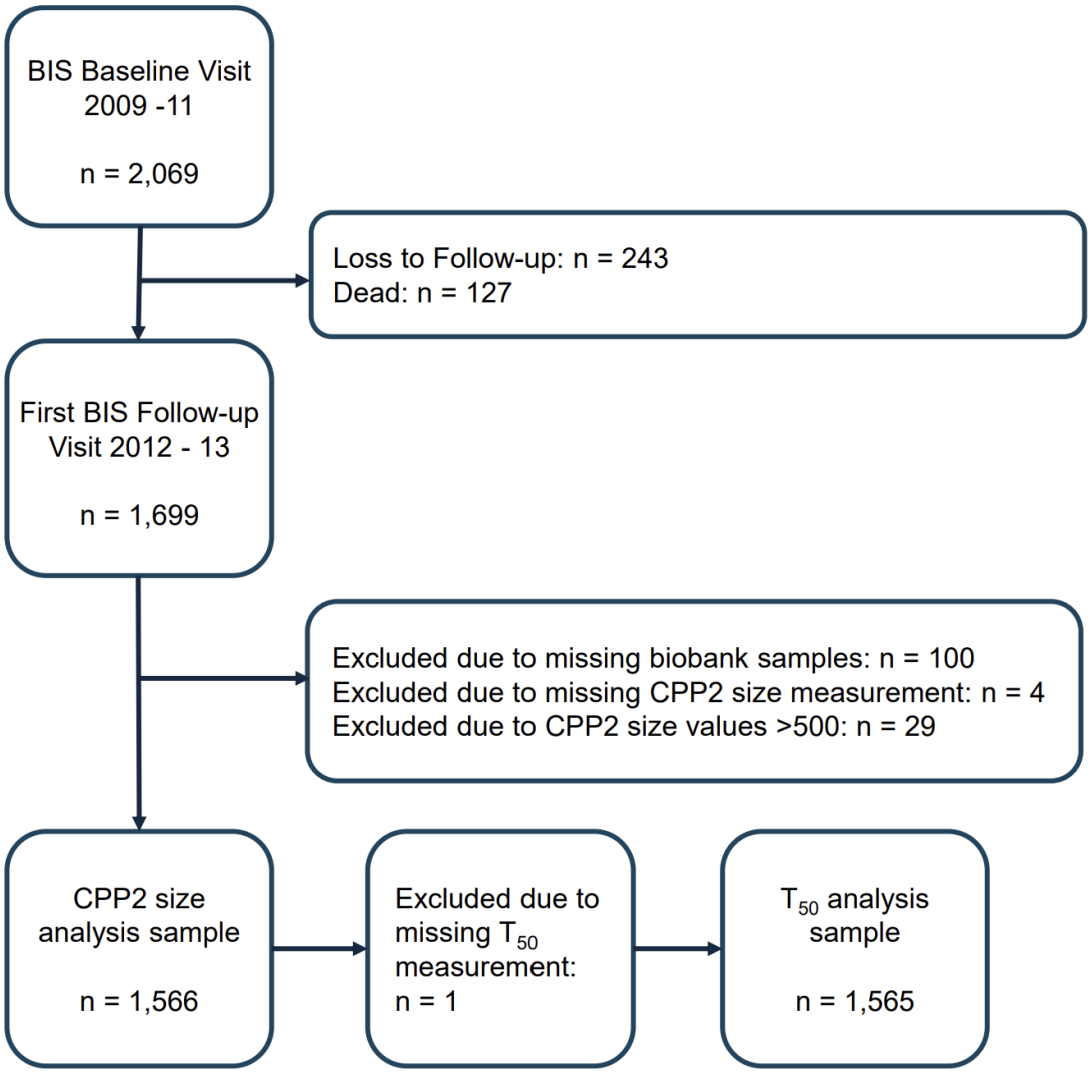
Phosphate Models									
Phosphate ≤1.19	Number of Events	401 / 777 (51.6%)				173 / 777 (22.3%)			
	HR (95% CI)	1.49 (1.35 – 1.65)	1.23 (1.10 – 1.38)	1.14 (1.04 – 1.26)	1.08 (0.98 – 1.19)	1.40 (1.19 – 1.64)	1.09 (0.91 – 1.31)	1.05 (0.91 – 1.22)	0.99 (0.85 – 1.14)
Phosphate >1.19	Number of Events	393 / 753 (52.2%)				167 / 753 (22.2%)			
	HR (95% CI)	1.38 (1.26 – 1.50)	1.15 (1.04 – 1.27)	1.16 (1.05 – 1.28)	1.11 (1.01 – 1.21)	1.49 (1.31 – 1.69)	1.22 (1.06 – 1.41)	1.09 (0.94 – 1.26)	1.03 (0.90 – 1.19)
Corrected Calcium Models									
Corr. Ca ≤2.37	Number of Events	393 / 768 (51.2%)				177 / 768 (23.1%)			
	HR (95% CI)	1.46 (1.32 – 1.61)	1.17 (1.05 – 1.31)	1.14 (1.03 – 1.25)	1.08 (0.98 – 1.18)	1.46 (1.26 – 1.69)	1.11 (0.94 – 1.31)	1.10 (0.95 – 1.26)	1.03 (0.90 – 1.19)
Corr. Ca >2.37	Number of Events	401 / 762 (52.6%)				163 / 762 (21.4%)			
	HR (95% CI)	1.40 (1.28 – 1.52)	1.20 (1.09 – 1.32)	1.15 (1.05 – 1.26)	1.12 (1.02 – 1.22)	1.44 (1.27 – 1.65)	1.22 (1.05 – 1.42)	1.03 (0.90 – 1.19)	1.00 (0.87 – 1.15)

Hazard Ratio (HR), 95% confidence interval (CI); estimated glomerular filtration rate (eGFR); corrected Calcium (Corr. Ca)
Model 1: adjusted for age and sex, *Sex-stratified model only for age

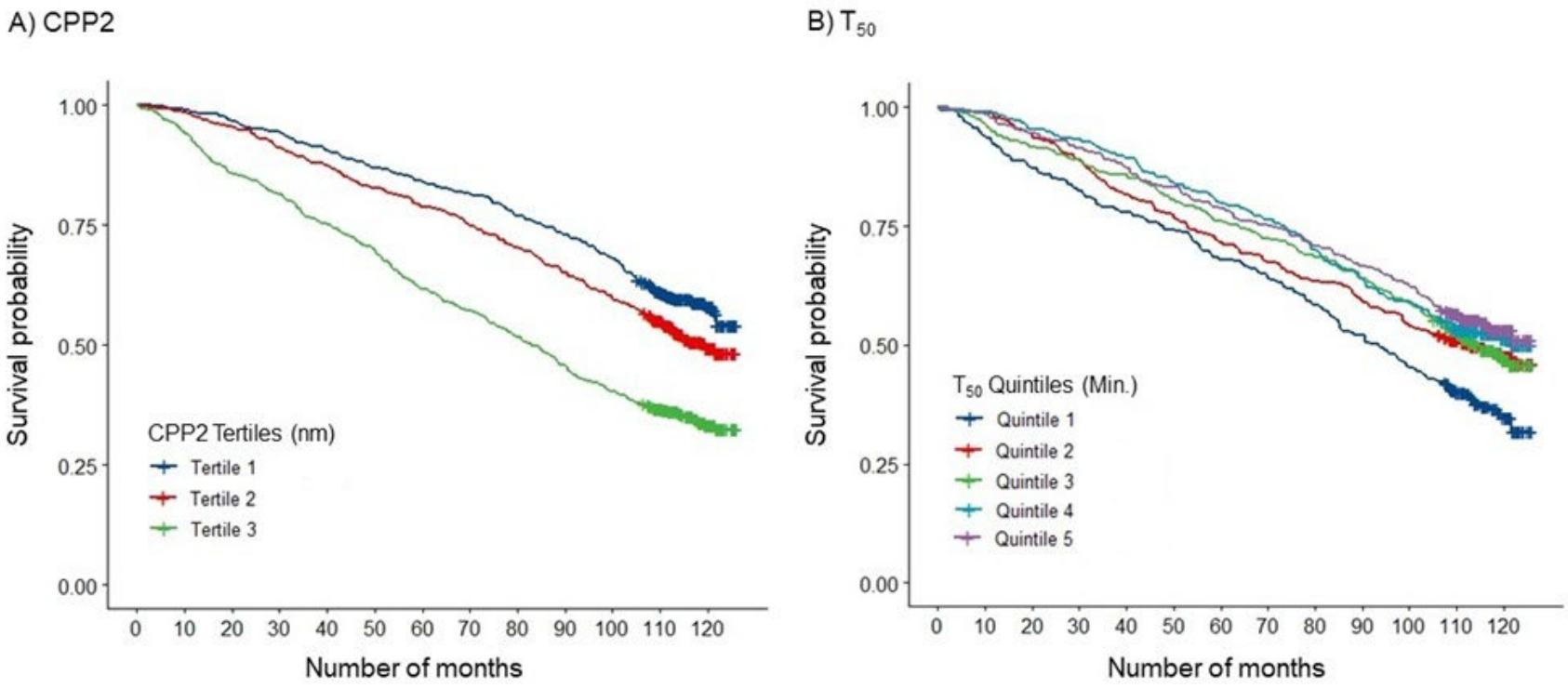
Supplementary Table 6. Crude and adjusted hazard ratios from Cox proportional hazard regression models for the association of serum phosphate with all-cause and cardiovascular mortality.

	All-cause Mortality HR (95% CI)				**Cardiovascular Mortality HR (95% CI)**			
	Number of Events	**Model**			**Number of Events**	**Model**		
		Crude	**Adj. 1**	**Adj. 2**		**Crude**	**Adj. 1**	**Adj. 2**
Serum Phosphate (mmol/l)	794 / 1530 (51.9%)	1.06 (0.78 – 1.43)	1.25 (0.92 – 1.69)	1.10 (0.80 – 1.51)	340 / 1530 (22.2%)	1.17 (0.74 – 1.85)	1.33 (0.85 – 2.10)	1.13 (0.70 – 1.80)

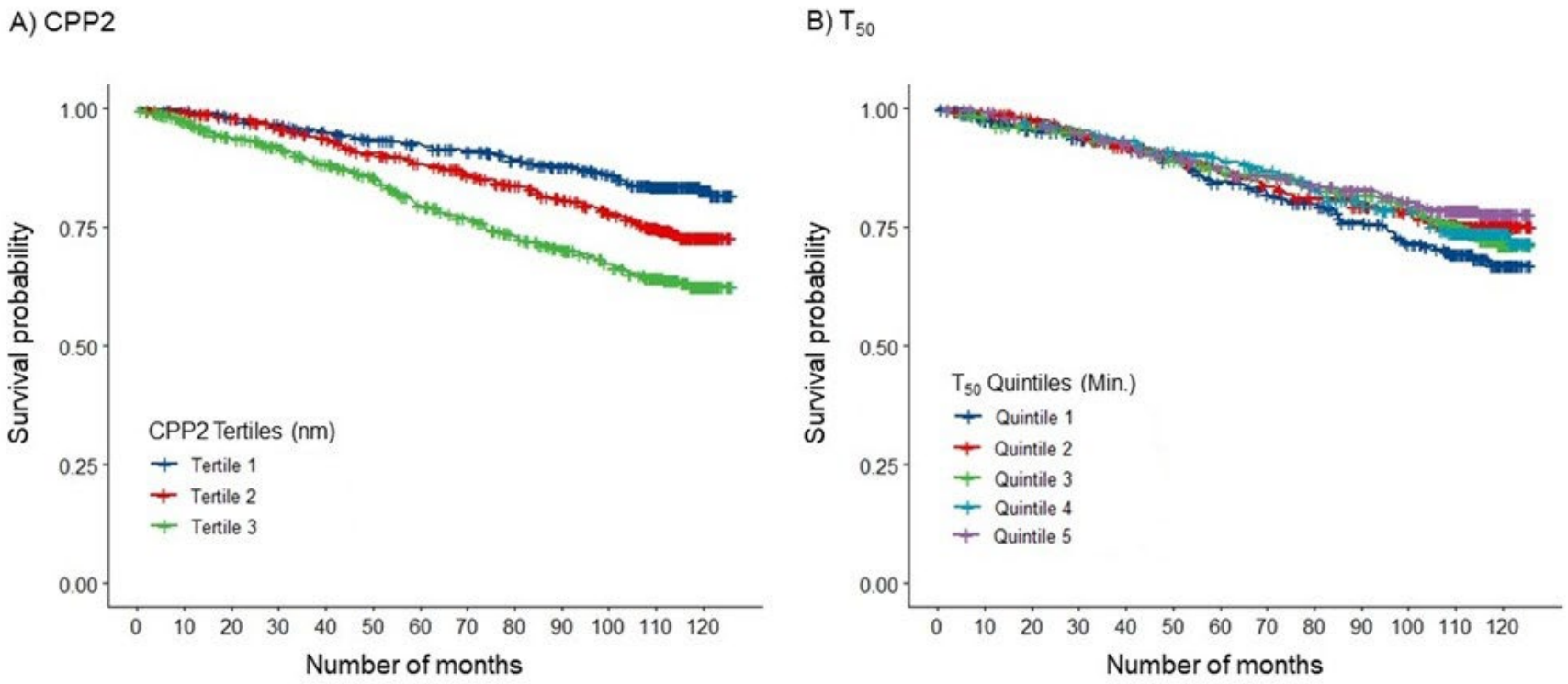
Hazard ratios (HR) and 95% confidence interval (CI)
Model 1: adjusted for age and sex
Model 2: adjusted for age, sex, smoking, systolic blood pressure, hypertension, diabetes, total blood cholesterol, BMI, CRP, eGFR, ACR, alcohol intake, lipid-lowering drugs, and history of CVD.



Supplementary Figure 1 . Flow-chart of the study.



Supplementary Figure 2. Kaplan Meier curves for all-cause mortality stratified by: A) CPP2 size tertiles and B) T_{50} quintiles. (T1 [90,163]; T2 [163,201]; T3 [201,464]); (Q1 [80,277]; Q2 [277, 313]; Q3 [313, 348]; Q4 [348, 384]; Q5 [384, 561]).



Supplementary Figure 3. Kaplan Meier curves for cardiovascular mortality stratified by: A) CPP2 size tertiles and B) T_{50} quintile.
(T1 [90,163]; T2 [163,201]; T3 [201, 464]); (Q1 [80,277]; Q2 [277,313]; Q3 [313,348]; Q4 [348,384]; Q5 [384,561]).