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Plasma klotho and cardiovascular disease in adults

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Abstract

OBJECTIVES—To determine whether plasma klotho, a recently discovered hormone that has been implicated in atherosclerosis, is related to prevalent cardiovascular disease in adults.

DESIGN—Cross-sectional.

SETTING—Population-based sample of adults residing in Tuscany, Italy.

PARTICIPANTS—One thousand and twenty-three men and women, aged 24–102, participating in the Invecchiare in Chianti (InCHIANTI) study.

MEASUREMENTS—Anthropometric measures, plasma klotho, fasting plasma total, high-density lipoprotein (HDL) cholesterol, triglycerides, glucose, creatinine, C-reactive protein. Clinical measures: medical assessment, diabetes mellitus, hypertension, coronary heart disease, heart failure, stroke, peripheral artery disease, cancer, chronic kidney disease. Logistic regression models were used to examine the relationship between plasma klotho and prevalent cardiovascular disease.

RESULTS—Of 1023 participants, 259 (25.3%) had cardiovascular disease. Median (25th, 75th percentile) plasma klotho concentrations were 676 (530, 819) pg/mL. Plasma klotho was correlated with age ($r = -0.14$, $P < 0.0001$), HDL cholesterol ($r = 0.11$, $P = 0.0004$), C-reactive protein ($r = -0.10$, $P = 0.0008$), but not systolic blood pressure, fasting plasma glucose, or renal function. Plasma klotho age-adjusted geometric means (95% Confidence Interval [C.I.]) were 626 (601, 658) in participants with cardiovascular disease and 671 (652, 692) pg/mL in those without cardiovascular disease ($P = 0.0001$). Adjusting for traditional cardiovascular risk factors (age, sex, smoking, total cholesterol, HDL cholesterol, systolic blood pressure, and diabetes), log plasma klotho was associated with prevalent cardiovascular disease (Odds Ratio per 1 standard deviation increase = 0.85, 95% C.I. 0.72, 0.99).

CONCLUSION—In community-dwelling adults, higher plasma klotho concentrations are independently associated with a lower likelihood of having cardiovascular disease.

Keywords

aging; atherosclerosis; cardiovascular disease; C-reactive protein; klotho

The aging suppressor gene *klotho* encodes a single-pass transmembrane protein that is predominantly expressed in the distal tubule cells of the kidney, parathyroid glands, and choroid plexus of the brain. The *klotho* gene was named after one of the three Fates in Greek mythology, the goddess who spins the thread of life. *Klotho* was originally identified in a mutant mouse strain that could not express klotho, developed multiple disorders resembling human aging, and had a shortened life span.¹ The aging phenotypes included atherosclerosis, endothelial dysfunction, decreased bone mineral density, sarcopenia, skin atrophy, and impaired cognition.^{2,3} In an atherosclerotic mouse model, the *in vivo* gene delivery of *klotho* protected against endothelial dysfunction.⁴ Overexpression of *klotho* in transgenic mice resulted in a significant extension of life span compared with wild-type mice.⁵

There are two forms of klotho, membrane and secreted, and each has different functions. Membrane klotho acts as an obligate co-receptor for fibroblast growth factor (FGF)-23, a bone-derived hormone that induces phosphate excretion into urine.⁶ Secreted klotho is involved in regulation of nitric oxide production in the endothelium,^{2,4} calcium homeostasis in the kidney,^{7,8} and inhibition of intracellular insulin and insulin-like growth factor-1 signaling.⁵ *Klotho* gene transcripts for a putative secreted form of klotho protein were described in 1998.⁹ In 2004, Imura and colleagues demonstrated that klotho protein was present in both human sera and cerebrospinal fluid.¹⁰ The relationship of circulating klotho with clinical phenotypes in human aging has not been studied because of the lack of a sensitive and reliable assay for measurement of secreted klotho protein in the blood. For example, it is not known whether low plasma klotho levels are associated with cardiovascular disease in humans. Recently, a sensitive and specific assay was developed for the measurement of soluble klotho in humans.¹¹

We hypothesized that low plasma klotho concentrations were independently associated with cardiovascular disease. To address this hypothesis, we measured plasma klotho levels in a large, population-based study of aging.

MATERIALS AND METHODS

Participants and Setting

The study participants consisted of men and women who participated in the Invecchiare in Chianti, "Aging in the Chianti Area" (InCHIANTI) study, conducted in two small towns in Tuscany, Italy. The rationale, design, and data collection have been described elsewhere, and the main outcome of this longitudinal study is mobility disability.¹² Briefly, in August 1998, 1299 people aged 65 years and older and 431 subjects from age strata 20–29, 30–39, 40–49, 50–59, and 60–64 years were randomly selected from the population registry of Greve in Chianti (pop. 11,709) and Bagno a Ripoli (pop. 4,704). Of 1,701 eligible subjects, 1,155 (90.1%) of people aged 65 years and older and 299 (69.4%) of people under age 65 agreed to participate. Participants received an extensive description of the study and participated after written, informed consent. The study protocol complied with the Declaration of Helsinki and was approved by the Italian National Institute of Research and Care on Aging Ethical Committee and by the Institutional Review Board of the Johns Hopkins University School of Medicine. This cross-sectional study of klotho and cardiovascular disease was conducted at the three-year follow-up visit from 2001–2003 (n = 1167) because of greater availability of archived plasma samples for analysis.

Clinical Evaluation

Demographic information and information on smoking and medication use were collected using standardized questionnaires. Smoking history was determined from self-report. Daily alcohol intake, expressed in gm/day, was determined based upon the European Prospective Investigation into Cancer and Nutrition food frequency questionnaire that had been validated in the Italian population. Education was recorded as years of school. All participants were examined by a trained geriatrician, and diseases were ascertained according to standard, pre-established criteria and algorithms based upon those used in the Women's Health and Aging Study for diabetes mellitus, coronary heart disease, heart failure, stroke, and cancer.¹³ Cardiovascular disease was defined as any one of the following: coronary heart disease, heart failure, stroke, and peripheral artery disease.

Systolic and diastolic blood pressures were calculated from the mean of three measures taken with a standard mercury sphygmomanometer during the physical examination. Weight was measured using a high-precision mechanical scale. Standing height was measured to the nearest 0.1 cm. Body mass index (BMI) was calculated as weight/height² (kg/m²). Mini-Mental State Examination (MMSE) was administered, and an MMSE score <24 was considered consistent with cognitive impairment.¹⁵ Chronic kidney disease was defined as estimated glomerular filtration rate of <60 mL/min/1.73 m² using the four-variable Modification of Diet in Renal Disease Study equation of Levey and colleagues.¹⁶

Laboratory Studies

Blood samples were collected in the morning after a 12-h fast. Aliquots of serum and plasma were immediately obtained and stored at -80° C. Plasma klotho was measured in 1023 (87.7%) participants who returned for the three-year follow-up visit. Soluble α -klotho was measured in plasma using a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) (Immuno-Biological Laboratories, Takasaki, Japan).¹¹ The minimum level of detectability of the assay is 6.15 pg/mL. The minimum level is below the plasma concentrations that were found in our study. The intra-assay and inter-assay coefficients of variation were 4.1% and 8.9% for klotho measurements in the investigator's (RDS) laboratory. The designation α -klotho is used to describe the original *klotho* gene and its product¹⁰ and to distinguish it from a homolog which was named β -klotho.¹⁷ In this paper, the term klotho will refer to α -klotho. Commercial enzymatic tests (Roche Diagnostics) were used for measuring serum total cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol concentrations. Low-density lipoprotein (LDL) cholesterol was calculated by the Friedewald formula.¹⁸ High sensitive C-reactive protein was measured using ELISA and a colorimetric competitive immunoassay (Calbiochem, San Diego, CA).

Statistical Analysis

Variables are reported as medians (25th, 75th percentiles) or as percentages. Spearman correlations were used to examine correlation between variables. Characteristics of subjects by tertile of plasma klotho were compared using Wilcoxon rank sum tests for continuous variables and chi-square tests for categorical variables. Logistic regression models were used to examine the relationship between plasma klotho and prevalent cardiovascular disease. Traditional risk factors for cardiovascular disease (age, sex, smoking, total cholesterol, HDL cholesterol, systolic blood pressure, diabetes) were entered as covariates into the multivariate logistic regression model, with additional models in which chronic kidney disease and C-reactive protein were added as covariates. Receiver operating characteristic curves^{19,20} were used to examine the relationship of plasma klotho, traditional risk factors, and other covariates with prevalent cardiovascular disease. All analyses were performed using SAS (v. 9.1.3, SAS Institute, Inc., Cary, NC) with a type I error of 0.05.

RESULTS

The characteristics of the participants in the InCHIANTI study at the three-year follow-up visit are shown overall and by tertile of plasma klotho in Table 1. Of the 1023 participants, 259 (25.3%) had cardiovascular disease. Higher plasma klotho was significantly associated with younger age, female sex, lower alcohol intake, never smoking or former smoking, higher cognition, higher HDL cholesterol, lower triglycerides, lower C-reactive protein, and lower prevalence of cardiovascular disease. There were no significant differences in education, body mass index, physical activity, fasting plasma glucose, total cholesterol, LDL cholesterol, systolic or diastolic blood pressure, or the prevalence of hypertension, coronary heart disease, heart failure, peripheral artery disease, stroke, diabetes mellitus, cancer, or chronic kidney disease across the tertiles of plasma klotho concentrations.

The Spearman correlations between plasma klotho (pg/mL) and other factors were as follows: age (years) ($r = -0.14$, $P < 0.0001$), total cholesterol (mg/dL) ($r = 0.01$, $P = 0.66$), HDL cholesterol (mg/dL) ($r = 0.11$, $P = 0.0004$), triglycerides ($r = -0.09$, $P = 0.003$), systolic blood pressure (mm Hg) ($r = -0.04$, $P = 0.26$), fasting plasma glucose (mg/dL) ($r = -0.019$, $P = 0.55$), eGFR (mL/min/1.73 m²) ($r = 0.04$, $P = 0.18$), and C-reactive protein (mg/L) ($r = -0.10$, $P = 0.0008$).

Multivariate logistic regression models were used to examine the relationship between plasma klotho and prevalent cardiovascular disease (Table 2). Plasma klotho was associated with cardiovascular disease in models adjusting for age, sex, smoking, total cholesterol, HDL cholesterol, systolic blood pressure, and diabetes (model 1) and additionally for eGFR (model 2). The odds ratio for plasma klotho and prevalent cardiovascular disease remained unchanged after addition of C-reactive protein, but the results were marginally significant ($P = 0.051$).

We used bootstrap resampling of the 1,023 subjects with 1,000 replications in order to examine receiver operating characteristic curves and the C-index for the three models above, with and without plasma klotho. The C-index is the percent of area under the receiver operating characteristic curve where 0.5 is no better than a guess and 1.0 is perfect correlation. For model 1, the C-index was 0.755 (95% C.I. 0.754, 0.756) for traditional cardiovascular risk factors compared with a C-index of 0.760 (95% C.I. 0.759, 0.762) when klotho was added to the model ($P < 0.0001$). For model 2, the C-index was 0.756 (95% C.I. 0.755, 0.757) for traditional cardiovascular risk factors and chronic kidney disease compared with a C-index of 0.762 (95% C.I. 0.760, 0.763) when klotho was added to the model ($P < 0.0001$). For model 3, the C-index was 0.762 (95% C.I. 0.761, 0.763) for traditional cardiovascular risk factors, chronic kidney disease, and C-reactive protein, compared with a C-index of 0.767 (95% C.I. 0.765, 0.768) when klotho was added to the model ($P < 0.0001$). For model 2, with the traditional cardiovascular risk factors, chronic kidney disease, and klotho, the C-index was 0.762 (95% C.I. 0.760, 0.763) compared to a C-index of 0.764 (95% C.I. 0.763, 0.765) when C-reactive protein was added to the model ($P < 0.0001$).

Discussion

The present study shows that plasma klotho is independently associated with cardiovascular disease in adults. The risk of cardiovascular disease was lower in adults who had higher plasma klotho concentrations. To our knowledge, this is the first study of plasma klotho and cardiovascular disease in humans. Klotho may be of special interest as a risk factor for cardiovascular disease in humans because atherosclerosis, oxidative stress, and endothelial dysfunction have been shown to be related to the level of expression of klotho in mice.^{1,2,4,5} The homology between the *klotho* gene in mouse and humans is extremely high; in humans,

the secreted form of klotho is more dominant than the membrane form.⁹ Genetic variants of *klotho* have been associated with coronary artery disease,^{21–23} stroke,^{24,25} and longevity^{25,26} in humans, but whether genetic variants of *klotho* are associated with differing levels of circulating klotho remains to be determined.

Klotho exerts pleiotropic effects upon the endothelium. The endothelium, a cell layer lining the entire vascular tree, has anti-inflammatory, anti-thrombotic, and vasodilatory properties which maintain blood flow while preventing thrombosis and leukocyte diapedesis.^{27,28} Endothelial cells maintain a relaxed vascular tone by releasing nitric oxide and prostacyclin, by controlling local angiotensin-II activity, and by regulating vascular permeability.²⁹ In addition, the endothelium regulates platelet and leukocyte adhesion and aggregation and thrombosis.²⁹ The loss of balanced endothelial regulation of blood vessel function is known as endothelial dysfunction, an early key event in the development of atherosclerosis.^{27,29} The characteristics of endothelial dysfunction include a reduced bioavailability of nitric oxide, impaired endothelium-dependent vasorelaxation, increased intracellular Ca^{2+} concentrations and endothelial permeability, increased generation of reactive oxygen species and expression of adhesion molecules, cytokines, and chemokines that facilitate leukocyte attachment to the endothelium and transmigration into the extracellular matrix.^{27,29,30} Thus, endothelial dysfunction constitutes an important initial step in the pathogenesis of atherosclerosis.

Circulating klotho plays a key role in regulating nitric oxide availability in the endothelium.^{2,4,31} Synthesis of nitric oxide and vasodilation are impaired in *klotho* mice, but endothelial function can be restored in *klotho* mice by parabiosis (anatomical connection of the circulation) with wild-type mice.⁴ In a rat model of multiple atherogenic risk factors, *in vivo* adenovirus-mediated *klotho* gene delivery improved endothelial function, increased nitric oxide production, reduced elevated blood pressure, and prevented medial hypertrophy and perivascular fibrosis.⁴

Circulating klotho maintains endothelial integrity and protects against vascular permeability. In endothelial cells, Ca^{2+} signals regulate the production and release of nitric oxide, permeability, proliferation, migration, and apoptosis.^{28,32} The transient receptor potential canonical-1 (TRPC-1) channel is involved in vascular endothelial growth factor (TGF)-mediated Ca^{2+} entry.^{33,34} Klotho maintains endothelial integrity by binding with both TRPC-1 and VEGFR-2. The complex is internalized in response to VEGF stimulation, thus regulating VEGF-mediated Ca^{2+} influx.³⁵ In klotho-deficient mice, the vascular endothelium is hyperpermeable because of enhanced apoptosis and decreased surface expression of vascular endothelial cadherin and hyperactivity of Ca^{2+} -dependent calpain/caspase-3.³⁶

Soluble klotho suppresses tumor necrosis factor- α -induced expression of adhesion molecules ICAM-1 and VCAM-1 in the endothelium.³⁶ Klotho-deficient mice display other characteristics that provide some clues to how klotho may affect the cardiovascular system. Klotho-deficient mice also show impaired ischemia-induced neovascularization,^{37,38} increased expression of plasminogen activator inhibitor-1,³⁹ and sinoatrial node dysfunction and early, unexpected cardiac death.⁴⁰ Interestingly, statins, which exert vascular protective effects, have been shown to inactivate the RhoA pathway and increase the expression of klotho mRNA.⁴¹

The results of the present study corroborate the negative correlation found between blood klotho levels and age in a study of healthy children and adults.¹¹ The previous study by Yamazaki and colleagues showed a significant negative correlation between creatinine and serum klotho, which suggested that serum klotho concentrations might be a possible marker

for renal function.¹¹ However, in the present study, conducted with a population-based sample in which about one-quarter of the participants had chronic kidney disease, there was no significant relationship between plasma klotho concentrations and estimated GFR. The present study does not support the idea that plasma klotho levels are a marker for renal function. Whether low circulating klotho levels are associated with more severe renal disease, such as end-stage renal disease, needs to be addressed in the future.

The present study showed that plasma klotho was positively correlated with HDL cholesterol. It has been hypothesized that there is a functional interrelationship between HDL cholesterol and klotho through insulin signaling, inhibition of apoptosis, or through other mechanisms.⁴² Genetic variants of klotho have been associated with HDL cholesterol²⁵ or with LDL cholesterol,⁴³ but as previously noted, it is unknown whether klotho polymorphisms are associated with differences in circulating klotho levels.

In this study, we used receiver operating characteristic curves to determine whether klotho provides information beyond that provided by established cardiac risk factors in relation to prevalent cardiovascular disease. Although the incremental increase in the C-index with the addition of plasma klotho was modest, a limitation of the present study is that the C-index was applied to prevalent cardiovascular disease. The C-index has been mainly applied in cardiovascular epidemiology to determine the added value of a biomarker for incident cardiovascular events, not prevalent cardiovascular disease. Prospective studies are needed to determine whether klotho may provide valuable information regarding the likelihood of cardiovascular disease in addition to that provided by traditional cardiovascular risk factors. The present study limited in that it is cross-sectional and involves prevalent cardiovascular disease, and further studies will be needed to evaluate klotho as a novel biomarker for incident cardiovascular events in addition to traditional risk factors.

The strengths of the present study include that it was a population-based sample of adults, that chronic diseases were carefully adjudicated, and that the data included conventional cardiovascular risk factors. The study is limited in that, as with any epidemiological study, there may be unmeasured confounding factors that could affect the relationship between plasma klotho and cardiovascular disease. Whether the findings from the present study, conducted in adults from the Tuscany region of Italy, can be generalized to other populations is not known. The cross-sectional design of the study can only demonstrate associations, as it was not possible with the size of the study cohort to show whether plasma klotho concentrations were predictive of incident cardiovascular disease.

In conclusion, this study is the first to show that adults with higher plasma klotho concentrations have a lower risk of cardiovascular disease. Future studies are needed to validate these findings in an independent study population and to determine whether plasma klotho levels are predictive of incident cardiovascular disease. Klotho may represent a potential target for new therapeutic interventions aimed at the prevention of cardiovascular disease.

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CONFLICT OF INTEREST

Financial Disclosures

Richard D. Semba: None

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Conflict of Interest Disclosures

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	Yes	No	Yes	No	Yes	No	Yes	No
Employment or Affiliation		X		X		X		X
Grants/Funds		X		X		X		X
Honoraria		X		X		X		X
Speaker Forum		X		X		X		X
Consultant		X		X		X		X
Stocks		X		X		X		X
Royalties		X		X		X		X
Expert Testimony		X		X		X		X
Board Member		X		X		X		X
Patents		X		X		X		X
Personal Relationship		X		X		X		X
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	Yes	No	Yes	No	Yes	No	Yes	No
Employment or Affiliation		X		X		X		X
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Honoraria		X		X		X		X
Speaker Forum		X		X		X		X
Consultant		X		X		X		X
Stocks		X		X		X		X
Royalties		X		X		X		X
Expert Testimony		X		X		X		X
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	Yes	No	Yes	No	Yes	No	Yes	No
Patents		X		X		X		X
Personal Relationship		X		X		X		X

Author Contributions

Richard D. Semba: Origination of hypothesis and study design, laboratory analysis, data analysis and interpretation, preparation of manuscript.

Anne R. Cappola: Data analysis and interpretation, preparation of manuscript.

Kai Sun: Primary data analysis, preparation of manuscript.

Stefania Bandinelli: Acquisition of subjects and data, data analysis and interpretation, preparation of manuscript.

Mansi Dalal: Laboratory analysis, preparation of manuscript.

Candace Crasto: Laboratory analysis, preparation of manuscript.

Jack M. Guralnik: Data analysis and interpretation, preparation of manuscript.

Luigi Ferrucci: Origination of hypothesis and study design, data analysis and interpretation, preparation of manuscript.

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Table 1
Demographic and health characteristics of 1023 participants in the InCHIANTI Study by tertiles of plasma klotho

Characteristic*	Overall	Plasma klotho, tertiles (pg/mL)			P
		< 586 (n = 340)	587 – 769 (n = 342)	> 770 (n = 341)	
Age (years)	73 (68, 79)	75 (69, 80)	72 (66, 78)	72 (64, 77)	<0.0001
Sex (%)	Male	47.9	46.5	40.2	0.04
	Female	52.1	53.5	59.8	
Education, years	5.0 (4.0, 8.0)	5.0 (4.0, 8.0)	5.0 (5.0, 8.0)	5.0 (5.0, 8.0)	0.13
Alcohol intake (g/d)	5.9 (0.2, 23.3)	8.4 (0.2, 23.9)	6.7 (0.2, 23.2)	5.2 (0.2, 13.8)	0.04
Smoking (%)	Never	50.9	56.0	58.7	0.05
	Former	32.7	28.5	28.1	
	Current	16.5	15.5	13.2	
Body mass index (kg/m ²)	26.0 (23.4, 28.5)	26.3 (23.6, 28.7)	26.0 (23.3, 28.6)	26.0 (23.1, 28.4)	0.62
Physical activity (%)	Inactive	27.4	18.4	20.2	0.12
	Low	45.3	50.9	51.0	
	Moderate- High	27.3	30.7	28.8	
Mini-Mental State Exam score <24 (%)	22.9	29.1	21.3	18.5	0.001
Fasting plasma glucose (mg/dL) (%)	99	77.3	77.9	75.8	0.81
	100–125	15.5	15.6	17.4	
	>125	7.2	6.5	6.8	
Total cholesterol (mg/dL)	214 (186, 243)	212 (182, 245)	218 (189, 243)	216 (185, 241)	0.37
High density lipoprotein cholesterol (mg/dL)	56 (47, 68)	54 (45, 66)	56 (49, 67)	58 (49, 69)	0.007
Low density lipoprotein cholesterol (mg/dL)	130 (105, 157)	127 (104, 159)	132 (108, 157)	129 (105, 156)	0.52
Triglycerides (mg/dL)	112 (82, 154)	116 (87, 162)	114 (83, 160)	107 (79, 142)	0.02
Ln C-reactive protein (mg/L)	1.93 (0.95, 4.09)	2.17 (1.11, 4.55)	1.90 (0.91, 4.23)	2.10 (0.86, 3.31)	0.007
C-reactive protein >3.0 mg/L (%)	33.8	38.3	32.7	29.7	0.03
Fibrinogen (mg/dL)	346 (304, 392)	350 (302, 398)	347 (304, 394)	342 (305, 383)	0.41
Estimated glomerular filtration rate (mL/min/1.73 m ²)	72.3 (60.5, 86.4)	71.4 (58.0, 86.8)	72.2 (60.9, 86.1)	73.9 (62.2, 86.9)	0.42
Systolic blood pressure (mm Hg)	145 (133, 160)	146 (133, 160)	145 (133, 160)	146 (133, 160)	0.95
Diastolic blood pressure (mm Hg)	81 (76, 87)	80 (75, 86)	81 (77, 86)	81 (77, 87)	0.09

Characteristic*	Overall	Plasma klotho, tertiles (pg/mL)			P
		< 586 (n = 340)	587 – 769 (n = 342)	> 770 (n = 341)	
Hypertension (%)	63.1	65.5	63.2	60.7	0.20
Coronary heart disease (%)	5.0	6.5	4.5	4.1	0.17
Heart failure (%)	6.1	6.8	7.0	4.4	0.20
Peripheral artery disease (%)	10.7	12.4	9.9	10.0	0.32
Stroke (%)	6.1	8.2	4.7	5.6	0.15
Cardiovascular disease (%)	25.3	32.7	23.1	20.2	0.0002
Diabetes mellitus (%)	11.6	14.1	9.4	11.4	0.28
Cancer (%)	7.5	9.1	7.3	6.2	0.14
Chronic kidney disease (%)	24.0	27.8	22.9	21.5	0.06

* Median (25th, 75th percentile) for continuous variables. Column percentages are shown for subcategories of smoking, physical activity, and fasting plasma glucose. Percentages are shown for specific conditions.

Table 2
Multivariate logistic regression models of the relationship between plasma klotho and cardiovascular disease

	Model 1	Model 2	Model 3
	Adjusted for age, sex, smoking, total cholesterol, HDL cholesterol, systolic blood pressure, and diabetes	Adjusted for age, sex, smoking, total cholesterol, HDL cholesterol, systolic blood pressure, diabetes, and chronic kidney disease	Adjusted for age, sex, smoking, total cholesterol, HDL cholesterol, systolic blood pressure, diabetes, chronic kidney disease, and C-reactive protein
O.R. (95% C.I.) per 1 SD increase of plasma log klotho ¹	0.85 (0.72, 0.99)	0.85 (0.72, 0.99)	0.85, (0.72, 1.00)

¹ One SD of log klotho (pg/mL) = 0.36