Aging and the risks of all-cause and cause-specific mortality among diabetics: a prospective cohort study

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Abstract

Background

Aging is an important driver for age-related diseases and death, but the evidence regarding the effects of aging on diabetics is limited. This study aims to evaluate the associations of aging with all-cause and cause-specific mortality among diabetics.

Methods

A total of 5,278 diabetics from the National Health and Nutrition Examination Survey 1999-2014 were included. The aging status was measured from different perspectives, including Phenotypic Age, Biological Age, telomere length, and Klotho concentration. Cox proportional hazards models were used to examine the relationships between aging and all-cause, cardiovascular disease (CVD), and cancer mortality. Mediation analysis was performed to elucidate the role of aging in associations of metformin with mortality.

Results

Over median follow-up for 7.3 years, 1,355 diabetics died. There was a positive and linear association of mortality with Phenotypic Age (hazard ratio (HR)$_{\text{all-cause}}$ 1.05, 95% confidence interval (CI) 1.05-1.06; HR$_{\text{CVD}}$ 1.05, 95%CI 1.04-1.07; HR$_{\text{cancer}}$ 1.05, 95%CI 1.04-1.07; all P<0.001) and Biological Age (HR$_{\text{all-cause}}$ 1.07, 95%CI 1.05-1.08; HR$_{\text{CVD}}$ 1.08, 95%CI 1.05-1.10; HR$_{\text{cancer}}$ 1.05, 95%CI 1.03-1.08; all P<0.001). Telomere length was inversely associated with all-cause mortality (tertile (T)3 vs. T1: HR 0.67, 95%CI 0.45-0.98; P_{trend}=0.036). The concentration of Klotho had a U-shaped relationship with mortality (T2 vs. T1: HR$_{\text{all-cause}}$ 0.62, 95%CI 0.43-0.88; HR$_{\text{CVD}}$ 0.48, 95%CI 0.26-0.86; HR$_{\text{cancer}}$ 0.47 95%CI 0.25-0.88). Additionally, metformin users had a lower HR for mortality compared to those without use (HR$_{\text{all-cause}}$ 0.64, 95%CI 0.56-0.73; HR$_{\text{CVD}}$ 0.51, 95%CI 0.37-0.70; HR$_{\text{cancer}}$ 0.65, 95%CI 0.44-0.95; all P<0.001), which was partly mediated by Phenotypic Age and Biological Age.

Conclusions

These findings suggested aging was a noteworthy risk factor of mortality for diabetics and therapies targeting anti-aging could be encouraged to halt the progression of diabetes.

Background

Diabetes, as the ninth major cause of death, is a worldwide public health concern, with considerable healthcare and economic burden (1). The number of diabetics has quadrupled in the past 3 decades and is projected to exceed more than 642 million by 2040 (2). Diabetics often suffer from complications and premature death (3). Statistically, the risk of death and cardiovascular events is 2 to 4 times higher among diabetics than in the general population (4). Hence, the identification of risk factors affecting the survival of diabetics may contribute to delaying premature mortality and its development.

Aging characterized by a progressive decline in homeostasis is generally accepted as the leading risk factor for age-related diseases and death (5). Nearly half of diabetics are older adults who are primarily responsible for the growing burden (6, 7). Moreover, diabetics usually develop a premature aging status with dysfunction of multiple systems (8). Compelling animal studies and clinical trials also revealed drugs or gene editing targeting aging improved diabetic outcomes (9, 10). Aging, though, was critical for diabetics, epidemiological evidence is limited. Previously, investigations explored the relationship between short telomere length and all-cause mortality among Chinese (11), Danish (12), and Italian diabetics (13), with the hazard ratio (HR) ranging from 1.87 to 3.45. However, some data showed that aging in the lung (14) and myocardium (15) was independent of telomere length, indicating the limited capacity of it to comprehensively reflect the aging status of the organ or organism. Recently, novel aging measures including multiple readily available clinical biomarkers were applied to capture body's homeostasis, such as Phenotypic Age and Biological Age. These markers were reported to well predict adverse outcomes. For example, a prospective cohort study in the general population showed that higher Phenotypic Age was positively associated with increased death risk, especially diabetes-caused mortality (HR=1.20) (16). However, studies focused on diabetics are lacking.

Therefore, we measured aging at multiple perspectives and conducted a prospective and representative cohort study to investigate the association between aging and all-cause and cause-specific mortality among U.S. diabetics based on the data from the National Health and Nutrition Examination Survey (NHANES) 1999-2014.

Methods

Study population

The NHANES is an ongoing national cross-sectional survey to assess the health and nutritional status in the U.S. The survey was approved by National Center for Health Statistics (NCHS) Ethics Review Board and all of the participants provide their written informed consent. In this study, all individuals with diabetes were included based on the data of NHANES from 1999 to 2014. Diabetes was defined by self-reported diagnosis, use of insulin or oral hypoglycemic medication, glycated hemoglobin (HbA_{1c}) level ≥6.5%, or fasting plasma glucose level ≥7.0 mmol/L (17). After excluding those who had missing information on any aging markers and/or mortality, and self-reported as pregnant, 5278 participants were enrolled in the current analysis.

Aging Markers Measurement

Phenotypic Age was calculated according to the method published previously (18). In brief, 9 aging-related variables, including chronological age, albumin, creatinine, glucose, Ln-C-reactive protein (CRP), lymphocyte percent, mean cell volume, red blood cell distribution width, alkaline phosphatase, and white blood cell count, were evaluated together based on the following equation.

$$\text{PhenotypicAge} = 141.50 + \frac{\text{Ln}[-0.00553 \times \text{Ln}(\exp\left(\frac{-1.51714 \times \exp(\text{xb})}{0.0076927}\right))]}{0.09165}$$

where: xb= -19.907−0.0336 × Albumin + 0.0095 × Creatinine + 0.1953 × Glucose + 0.0954 × LnCRP−0.0120 × Lymphocyte Percent + 0.0268 × Mean Cell Volume + 0.3306 × Red Cell Distribution Width + 0.00188 × Alkaline Phosphatase + 0.0554 × White Blood Cell Count + 0.0804× Chronological Age

Biological Age was calculated using the methods proposed by Klemera and Doubal (19). In this study, 8 biomarkers (CRP, serum creatinine, HbA_{1c}, serum albumin, serum total cholesterol, serum urea nitrogen, serum alkaline phosphatase, and systolic blood pressure) were estimated based on the following formulas (1-4) (20). The values *j* and *i* represent the number of biomarkers and samples respectively. The values *k*, *q*, and *s* are the regression slope, intercept, and the root means squared error of a biomarker regressed on chronological age, respectively. The value r_j^2 represents the variance explained by regression chronological age on biomarkers.

$$(1)\mathrm{BA}_E = \frac{\sum_{j=1}^{m}\left(x_j - q_j\right)\left(\frac{k_j}{s_j^2}\right)}{\sum_{j=1}^{m}\left(\frac{k_j}{s_j}\right)^2} \quad (2) r_{char} = \frac{\sum_{j=1}^{m}\frac{r_j^2}{\sqrt{1-r_j^2}}}{\sum_{j=1}^{m}\frac{r_j}{\sqrt{1-r_j^2}}}$$

$$(3) s_{BA}^2 = \frac{\sum_{j=1}^{n}\left(\left(\mathrm{BA}_{Ei} - \mathrm{CA}_i\right) - \frac{\sum_{i=1}^{n}\left(\mathrm{BA}_{Ei} - \mathrm{CA}_i\right)}{n}\right)^2}{n} - \left(\frac{1 - r_{char}^2}{r_{char}^2}\right) \times \left(\frac{\left(\mathrm{CA}_{max} - CA_{min}\right)^2}{12m}\right)$$

$$\mathrm{BA}_{EC} = \frac{\sum_{j=1}^{m}\left(x_j - q_j\right)\left(\frac{k_j}{s_j^2}\right) + \frac{\mathrm{CA}}{s_{BA}^2}}{\sum_{j=1}^{m}\left(\frac{k_j}{s_j}\right)^2 + \frac{1}{s_{\mathrm{BA}}^2}}$$

(4)

Additionally, markers of premature aging were defined as Phenotypic/Biological Age Acceleration and ΔPhenotypic/Biological Age (16). Phenotypic/Biological Age Acceleration was the residual resulting from a linear model when regressing Phenotypic/Biological Age on chronological age. ΔPhenotypic/Biological Age represented the value of Phenotypic/Biological Age minus chronological age.

Measurement of telomere length (NHANES 1999-2002) has been reported elsewhere (21). Briefly, blood samples were collected and determined by quantitative polymerase chain reaction (qPCR) assays to assess e T/S ratio). Klotho (NHANES 2007-2014) was

determined by a commercially available ELISA kit (IBL International, Japan). All sample analyses were performed in duplicate strictly following the manufacturer's instruction and all results were checked to meet the laboratory's standardized criteria for acceptability before being released for reporting.

Mortality Ascertainment

All participants from NHANES 1999-2014 were linked to death records in the National Death Index up to 31 December 2015. Follow-up duration is defined as the time from participating in the NHIS to death for decedents or to the censoring date for survivors. Mortality outcomes in the current study included all-cause, cardiovascular disease (CVD)-cause, and cancer-cause determined by ICD-10 codes recorded in NHANES.

Statistical analysis

All analyses were performed with SAS (version 9.4) or R (version 3.6.3) and accounted for the complex sampling design of the NHANES according to the analytic guidelines. We used χ^2 tests, nonparametric tests, and *t*-tests to assess the baseline characteristics of the participants by life or death status. Continuous variables were presented as mean ± standard error (SE) or median (interquartile range), and categorical variables were presented as n (%). Cox proportional hazards models were applied to estimate hazard ratio (HR) and 95% confidence interval (CI) for the associations of aging markers with all-cause and cause-specific mortality, with months of follow-up as the time scale. Aging markers were categorized into three tertiles (T1, T2, and T3) based on the distribution. The T/S ratio and Klotho were Ln-transformed (continuous). Model 1 was adjusted for age (<65 or ≥65 years), sex (male or female), race/ethnicity (Mexican American, other Hispanic, non-Hispanic White, non-Hispanic Black, or others), marital status (married/cohabiting, widowed/divorced/separated, or never married), BMI (<25.0, 25.0-29.9, or ≥30.0 kg/m^2), physical activity (moderate or vigorous), drinking alcohol status (ever or never), and Ln-cotinine concentration (continuous). Model 2 was further adjusted for triglyceride (continuous), medication use (insulin/pills or no), CVD (yes or no), cancer (yes or no), and hypertension (yes or no). The missing data of covariates were imputed with median values. The trend test across increasing exposure groups was calculated using integer values (1, 2, and 3). The dose-response relationships between aging markers and mortality were assessed by restricted cubic spline regression with 3 knots placed at the 25th, 50th, and 75th percentile.

We also estimated the associations of metformin use (ever or never) with mortality by the Cox proportional hazards model, and subgroup analysis was stratified by HbA_{1c} level (<6.5% or ≥6.5%). Mediation analyses were conducted to explore whether the use of metformin reduced mortality by anti-aging with an R package (mediation). The mediated proportion referred to the average mediation effect between metformin use attributed to mortality changes relative to the total effect. *P*-value for mediated proportion was obtained from the quasi Bayesian Monte Carlo simulation 2000 times.

Several sensitivity analyses were also performed. First, participants with a diagnosis of diabetes before the age of 20 years were excluded. Second, the deaths within 1 year of follow-up were excluded to reduce the potential reverse causation bias. Third, multiple imputations was used for covariates with missing values. Fourth, given the diabetes status, HbA_{1c} level (<6.5% or ≥6.5%) and duration of diabetes (<3, 3–10, or >10

years) were further adjusted. Fifth, considering other demographic confounding factors, we further adjusted for education levels (under high school, high school or equivalent, or above high school) and family income-poverty ratio (0–1.0, 1.0–3.0, or >3.0). Finally, some potential mediators were also further adjusted, including HDL-cholesterol, total cholesterol, and HOMA of insulin resistance.

Results

During median follow-up for 7.3 years, 1,355 deaths were documented among 5,278 diabetics. Based on the live or death status of the study population, baseline demographic was provided in Table 1. The deaths were older, more likely to be Non-Hispanic White and widowed or divorced or separated, and were less likely to be obese, and had less frequently drinking alcohol. Those who died had a longer duration of diabetes, and a high percentage of anti-diabetic medication or insulin use but had a lower percentage of metformin use. The deaths had a lower median concentration of Klotho (0.76 vs. 0.82 pg/mL), a shorter median telomere length (0.88 vs. 0.97), and an older mean Phenotypic Age (74.55 vs. 57.88 years) and Biological Age (68.39 years vs. 55.05 years).

Table 1
Baseline characteristics of diabetics by life or death status in NHANES 1999-2014.

Characteristics	Alive (3923)	Dead (1355)	*P* value
Age, years			<0.001
<65	2533 (70.55)	381 (35.84)	
≥65	1390 (29.45)	974 (64.16)	
Gender			0.058
Male	1984 (51.17)	768 (55.08)	
Female	1939 (48.83)	587 (44.92)	
Race/ethnicity			<0.001
Mexican American	912 (9.99)	264 (5.44)	
Other Hispanic	375 (6.39)	56 (4.51)	
Non-Hispanic White	1340 (60.60)	659 (70.89)	
Non-Hispanic Black	1012 (15.14)	326 (13.92)	
Other race/multiracial	284 (7.87)	50 (5.24)	
Marital status			<0.001
Married/cohabiting	2428 (65.83)	664 (51.51)	
Widowed/divorced/separated	1092 (24.41)	582 (42.05)	
Never married	371 (9.76)	81 (6.44)	
BMI, kg/m^2			<0.001
<25.0	456 (10.75)	250 (19.70)	
25-29.9	1107 (26.07)	430 (30.24)	
≥30.0	2277 (63.19)	564 (50.07)	
Physical activity			<0.001
Moderate	3180 (78.14)	1255 (91.80)	
Vigorous	743 (21.86)	100 (8.20)	
Drinking alcohol status			0.007
Ever	2295 (66.27)	726 (58.81)	
Never	1349 (33.73)	522 (41.19)	
Medication use			<0.001
Insulin use	2906 (80.81)	806 (88.25)	

Characteristics	Alive (3923)	Dead (1355)	*P* value
No	436 (19.99)	99 (11.74)	
Duration of diabetes, years			<0.001
≤3	735 (29.54)	158 (19.37)	
3-10.0	805 (29.41)	191 (24.56)	
>10	1162 (41.05)	497 (56.08)	
Serum cotinine, ng/mL	53.1 ± 3.16	49.67 ± 4.41	0.551
Triglyceride, mmol/L	2.12 ± 0.07	2.19 ± 0.06	0.488
CVD			<0.001
Yes	785 (18.59)	596 (45.55)	
No	3091 (81.41)	742 (54.45)	
Cancer			<0.001
Yes	428 (13.02)	269 (21.04)	
No	3467 (86.98)	1082 (78.96)	
Hypertension			<0.001
Yes	2478 (61.58)	950 (70.13)	
No	1431 (38.42)	398 (29.87)	
Metformin use			<0.001
Ever	1077 (37.40)	357 (26.99)	
Never	1709 (62.60)	989 (73.01)	
Klotho, ng/mL	0.82 (0.67, 1.02)	0.76 (0.60, 1.00)	0.027
Mean T/S ratio	0.97 (0.85, 1.13)	0.88 (0.77, 1.05)	0.001
Phenotypic Age, years	57.88 ± 0.38	74.55 ± 0.58	<0.001
Biological Age, years	55.05 ± 0.40	68.39 ± 0.54	<0.001
Continuous variables were presented as mean ± SE or median (interquartile range). Categorical variables were presented as numbers (percentages). All estimates accounted for complex survey designs.			

Table 2 presented the associations of 4 aging markers with all-cause, CVD, and cancer mortality. Each 1-year increase in Phenotypic Age increased the risk of all-cause, CVD, and cancer mortality by 5% (all P<0.001). Similarly, each 1-year increase in Biological Age was associated with an increase in all-cause, CVD, and cancer mortality (7%, 8%, and 5%, respectively, all P<0.001). Premature aging markers were also positively associated with all-cause, CVD, and cancer mortality (Supplementary Table 1). Additionally, for each unit increase in [illegible] all-cause mortality decreased by 47% (HR 0.53,

95%CI 0.29-0.97), consistent with the quantile analysis. The middle concentration of Klotho was associated with a lower risk of all-cause, CVD, and cancer mortality (T2 vs. T1: $HR_{all-cause}$ 0.62, 95%CI 0.43-0.88; HR_{CVD} 0.48, 95%CI 0.26-0.86; HR_{cancer} 0.47, 95%CI 0.25-0.88).

Table 2
HR (95% CI) for all-cause and cause-specific mortality based on aging markers among diabetics.

	All-cause mortality		CVD mortality		Cancer mortality	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
Phenotypic Age (years)						
Deaths/total	1251/4003		320/3072		208/2960	
Continuous	1.05 (1.05, 1.06)	1.05 (1.05, 1.06)	1.06 (1.05, 1.07)	1.05 (1.04, 1.07)	1.05 (1.04, 1.07)	1.05 (1.04, 1.07)
T1	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
T2	2.28 (1.56, 3.33)	2.13 (1.43, 3.17)	1.59 (0.71, 3.55)	1.26 (0.53, 3.01)	1.39 (0.52, 3.70)	1.29 (0.49, 3.43)
T3	6.28 (4.29, 9.20)	5.45 (3.62, 8.20)	3.88 (1.60, 9.40)	2.73 (1.02, 7.29)	4.02 (1.66, 9.72)	3.51 (1.43, 8.63)
P_{trend}	<0.001	<0.001	<0.001	0.004	<0.001	0.001
Biological Age (years)						
Deaths/total	1204/3861		311/2968		198/2855	
Continuous	1.07 (1.06, 1.08)	1.07 (1.05, 1.08)	1.09 (1.06, 1.11)	1.08 (1.05, 1.10)	1.06 (1.04, 1.09)	1.05 (1.03, 1.08)
T1	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
T2	2.20 (1.61, 3.01)	2.00 (1.46, 2.75)	1.93 (1.19, 3.14)	1.69 (0.98, 2.90)	2.11 (0.79, 5.65)	1.88 (0.71, 4.96)
T3	4.47 (2.98, 6.71)	3.85 (2.62, 5.65)	6.40 (2.60, 15.72)	4.91 (1.87, 12.90)	2.97 (0.90, 9.83)	2.20 (0.66, 7.38)
P_{trend}	<0.001	<0.001	<0.001	0.003	0.055	0.152
Mean T/S ratio						
Deaths/total	432/905		115/588		56/529	
Continuous	0.49 (0.25, 0.95)	0.53 (0.29, 0.97)	0.32 (0.09, 1.11)	0.36 (0.11, 1.14)	0.31 (0.05, 1.90)	0.34 (0.04, 2.68)
T1	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
T2	0.68 (0.48, 0.95)	0.70 (0.50, 0.97)	0.59 (0.31, 1.13)	0.55 (0.26, 1.15)	0.70 (0.28, 1.72)	0.80 (0.32, 2.00)
T3	0.63 (0.42, 0.95)	0.67 (0.45, 0.98)	0.70 (0.37, 1.33)	0.74 (0.42, 1.32)	0.48 (0.16, 1.48)	0.54 (0.15, 1.97)

	All-cause mortality		CVD mortality		Cancer mortality	
P_{trend}	0.026	0.036	0.190	0.239	0.188	0.343
Klotho (ng/mL)						
Deaths/total	286/2548		60/2322		69/2331	
Continuous	0.66 (0.43, 1.01)	0.69 (0.46, 1.04)	0.51 (0.16, 1.64)	0.53 (0.16, 1.70)	0.87 (0.29, 2.58)	0.87 (0.31, 2.47)
T1	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
T2	0.62 (0.45, 0.86)	0.62 (0.43, 0.88)	0.49 (0.28, 0.88)	0.48 (0.26, 0.86)	0.47 (0.24, 0.92)	0.47 (0.25, 0.88)
T3	0.78 (0.55, 1.12)	0.81 (0.56, 1.17)	0.84 (0.55, 1.30)	0.84 (0.53, 1.34)	0.85 (0.40, 1.82)	0.85 (0.40, 1.83)
P_{trend}	0.152	0.219	0.435	0.798	0.640	0.651

Model 1: adjusted for age (<65 or ≥65), sex (male or female), race/ethnicity (Mexican American, other Hispanic, non-Hispanic White, non-Hispanic Black, or other), marital status (married/cohabiting, widowed/divorced/separated, or never married), BMI (<25.0, 25.0-29.9, or ≥30.0 kg/m^2), physical activity (moderate or vigorous), drinking alcohol status (ever or never), and Ln-cotinine concentration (continuous). Model 2: further adjusted (from Model 1) for triglyceride (continuous), medication use (insulin/pills or no), CVD (yes or no), cancer (yes or no), and hypertension (yes or no). Deaths/total, the ratio of the number of deaths to the total participants; T, tertile.

A dose-response function showed Phenotypic and Biological Age had a linear relationship with all-cause, CVD, and cancer mortality (all *P* linearity <0.001). Telomere length also showed a linear relationship with all-cause (*P* linearity 0.015), CVD (*P* linearity 0.152), and cancer (*P* linearity 0.037) mortality. A U-shaped correlation was observed between Klotho and all-cause (*P* nonlinear 0.002), CVD (*P* nonlinear 0.502), and cancer (*P* nonlinear 0.014) mortality (Fig. 1 and Supplementary Fig. 1).

In the sensitivity analyses, similar results were observed after excluding participants with a diagnosis of diabetes before the age of 20 (Supplementary Table 2), imputing the missing data of covariates with multiple imputations (Supplementary Table 4), or further adjusting for HbA_{1c} level and the duration of diabetes (Supplementary Table 5). When excluding the deaths within 1 year of follow-up, further adjusting for education levels and family income-poverty ratio, or further adjusting for HDL-cholesterol, total cholesterol, and HOMA of insulin resistance, the results did not materially change (Supplementary Table 3, 6, and 7).

Furthermore, we investigated the relationship of metformin with mortality among diabetics. Metformin users had a lower HR for all-cause, CVD, and cancer mortality compared to non-use ($HR_{all-cause}$ 0.64, 95%CI 0.56-0.73; HR_{CVD} 0.51, 95%CI 0.37-0.70; HR_{cancer} 0.65, 95%CI 0.44-0.95) (Table 3). Consistently, the use duration of metformin was negatively associated with mortality (Supplementary Table 8). Such associations were also presented regardless of whether blood glucose was well controlled. Further, mediation analysis showed that through decreasing Phenotypic Age, metformin use reduced all-cause (mediated proportion=26.52%, *P*<0.001), and CVD mortality (mediated proportion=14.42%, P=0.018). Among people with HbA_{1c} greater than 6.5

percent, Phenotypic Age also mediated this association of metformin use with all-cause (mediated proportion=34.18%, *P*<0.001), and CVD mortality (mediated proportion=26.22%, *P*=0.048). Biological Age mediated the effect of metformin use on all-cause mortality in total people and people with HbA_{1c} greater than 6.5 percent, and the mediated proportion was 14.68% and 19.61%, respectively (Fig. 2 and Supplementary Fig. 2).

Table 3
HR (95% CI) for all-cause and cause-specific mortality based on the use of metformin stratified by HbA1c level among diabetics in NHANES 1999-2014.

	Total population	**HbA_{1c}<6.5%**	**HbA_{1c}≥6.5%**
	metformin vs. no metformin	metformin vs. no metformin	metformin vs. no metformin
All-cause mortality			
Deaths/total	1346/5266	418/1643	928/3623
Model 1	0.70 (0.61, 0.80)	0.59 (0.44, 0.80)	0.74 (0.63, 0.87)
Model 2	0.64 (0.56, 0.73)	0.57 (0.42, 0.77)	0.65 (0.56, 0.77)
CVD mortality			
Deaths/total	340/4260	106/1331	234/2929
Model 1	0.57 (0.41, 0.78)	0.49 (0.27, 0.89)	0.60 (0.41, 0.88)
Model 2	0.51 (0.37, 0.70)	0.43 (0.25, 0.75)	0.54 (0.36, 0.81)
Cancer mortality			
Deaths/total	226/4146	80/1305	146/2841
Model 1	0.70 (0.48, 1.04)	0.49 (0.22, 1.12)	0.88 (0.55, 1.41)
Model 2	0.65 (0.44, 0.95)	0.44 (0.20, 0.99)	0.81 (0.51, 1.27)

Model 1: adjusted for age (<65, or ≥65 years), sex (male or female), race/ethnicity (Mexican American, other Hispanic, non-Hispanic White, non-Hispanic Black, or other), marital status (married/cohabiting, widowed/divorced/separated, or never married), BMI (<25.0, 25.0-29.9, or ≥30.0 kg/m^2), physical activity (moderate or vigorous), drinking alcohol status (ever or never), and Ln-cotinine concentration (continuous). Model 2: further adjusted (from Model 1) for triglyceride (continuous), medication use (insulin/pills or no), CVD (yes or no), cancer (yes or no), hypertension (yes or no). Deaths/total, the ratio of the number of deaths to the total participants.

Discussion

In the current prospective cohort study of the U.S. population with diabetes, we found that aging-related makers were associated with mortality. Specifically, Phenotypic and Biological Age showed positive associations with all-cause, CVD, and cancer mortality, higher telomere length was associated with lower all-cause mortality, and Klotho had a U-shaped relationship with all-cause, CVD, and cancer mortality. Further,

metformin decreased all-cause, CVD, and cancer mortality risk. More importantly, such associations were partly mediated by Phenotypic Age and Biological Age. These findings suggested that the associations of aging with mortality were significant, and anti-aging was an important approach to reduce death among diabetics.

Previously, a meta-analysis of 17 cohorts with 5575 diabetics and 6439 control subjects showed shorter telomere length in diabetes (22). An inverse association was noted between telomere length and mortality among Chinese, Danish, and Italian diabetics (HR=1.87-3.45) (23). Consistent findings were also pronounced in our analysis among the U.S. diabetics. Telomere length is accepted as an indicator of cellular aging. Interestingly, removing aging cells or delaying their formation in mice can improve diabetes progress and its complications (10, 24). However, the aging process of mouse cardiomyocytes and human lungs is only linked to the telomere-associated DNA damage, without significant telomere shortening detected (14, 15). The above results may be attributed to the limited role of telomere in interpretation at organs or the whole body level. Importantly, diabetics often develop multiple organ disorders and die by complications. Hence, more comprehensive indicators which can represent the body's status were needed.

Phenotypic and Biological Age incorporated composite clinical biomarkers, such as inflammation (e.g. CRP), immunity (e.g. number of immune cells), and organ function (e.g. albumin and serum urea nitrogen) (25). They are useful to identify the unhealthy condition of patients caused by multiple organ disorders. Herein, we found that Phenotypic Age and Biological Age were significantly associated with all-cause and cause-specific mortality among diabetics. Moreover, we defined premature aging status based on chronological age and found that diabetics who appeared older than expected physiologically had a higher death risk. The previous study also reported similar associations of Phenotypic and Biological Age with all-cause mortality, but in the general population (HR=1.09, 1.10, respectively). Notably, among the disease-specific mortality, the risk of diabetes-caused mortality increased mostly (HR=1.20) (16). These researches indicated the importance of the whole body's aging for death among diabetics.

On the other hand, as the aging-related molecule, Klotho has been reported to be involved in the aging process for over 30 years, where it regulates phosphate homeostasis, insulin, and Wnt signaling (26). Interestingly, in our study population, Klotho concentration had a U-shaped relationship with all-cause, CVD, and cancer mortality. Previous epidemiological studies in the old or chronic kidney disease population revealed that Klotho concentration was negatively associated with death (27, 28). Indeed, overexpression of soluble Klotho up-regulated fibroblast growth factors-23 levels, which may lead to hypovitaminosis D (29). More importantly, a recent prospective cohort study including 6,329 diabetics reported that the level of serum 25-Hydroxyvitamin D was negatively associated with all-cause and cause-specific mortality (30). These studies supported our findings that circulating Klotho may have a U-shaped relationship with mortality risk in diabetics. Collectively, from many perspectives, we observed the effect of aging on promoting diabetics' death.

Metformin was first introduced to the world in 1957, as an anti-hyperglycemic agent. Consistent with previous studies, we found that metformin users had a lower risk of all-cause and cause-specific mortality, which were positively correlated with the number of days taken among diabetics. Recently, accumulating evidence has revealed the gerotherapeutic effect of metformin on lowering the incidence of multiple age-related diseases and all-cause mortality in diabetics (31). Aroor et al. also indicated that the anti-aging function of metformin

is possibly independent of its effect on diabetes control (32). Hence, we further investigated whether anti-aging was involved in the role of metformin in preventing mortality among diabetics. We found that metformin use decreased mortality risk among diabetics, regardless of whether HbA_{1C} was well controlled. Of note, our mediation analyses showed that metformin reduced mortality partly by decreasing Biological Age and Phenotypic Age, emphasizing the importance of anti-aging. Mechanism-related studies in multiple models have elucidated metformin participated in various pathways of aging, including deregulated nutrient sensing, altered intercellular communication, genomic instability, and loss of proteostasis (33, 34). Therefore, treatments targeting anti-aging are greatly promising in improving the mortality of diabetics. Some interventions have been well validated in animal or cells models (35). For example, senolytic drugs (e.g. ABT263) improved glucose tolerance and insulin sensitivity in diabetes mice by reducing senescent cell burden (10). Another combination senolytic agents, dasatinib and quercetin (DQ), offered a good therapeutic effect on mice with age-related diseases (36). In particular, preliminary clinical trials presented the positive effects of DQ on patients with idiopathic pulmonary fibrosis and diabetic kidney disease (9, 37). The current study provided evidence that anti-aging was effective to delay the progression of diabetes and mortality.

This study has some strengths. Firstly, the study was a prospective cohort study based on data from a large nationally representative survey among U.S. diabetics. Secondly, the associations reported in this study were relatively robust by adjustment for a variety of confounders and several sensitivity analyses. Finally, we examined the relationship between aging and death outcomes among diabetics from multiple perspectives. There are also limitations. Firstly, considering the cross-sectional nature of the NHANES data, the time-varying changes in aging markers could not be investigated. Secondly, the study lacked further information on the severity of diabetes, though adjusting for diabetes medication use, duration of diabetes, HbA_{1c} levels, and some self-reported comorbidities. Thirdly, unmeasured confounders and measurement errors may bias our analyses. Finally, whether anti-aging is involved in the effect of metformin on mortality still needs further research. In addition, we only analyzed the role of one anti-aging drug in mortality, which limited the interpretation of our conclusions.

Conclusions

In summary, our results of nationally representative samples from a prospective cohort study showed significant associations of aging with total and cause-specific mortality among diabetics. Moreover, we observed the improvement in mortality by metformin use among diabetics and further highlighted the mediating effects of aging. These findings suggested that aging accelerated death among diabetics and anti-aging treatments as a promising approach for diabetics.

Abbreviations

CVD: cardiovascular disease; HR: hazard ratio; CI: confidence interval; T: tertile; HbA_{1c}: glycated hemoglobin; CRP: C-reactive protein; SE: standard error; NCHS: National Center for Health Statistics.

Declarations

The authors thank all participants and all investigators.

Author's contributions

Li Chen and Tianqi Tan conducted analyses. Li Chen, Ying Zhao, and Huimin Chen wrote the draft of the article. Ping Yao and Yuhan Tang conceived of the study design. All authors contributed to the interpretation of the results and critical revision of the manuscript for important intellectual content and approved the final version of the manuscript. Yuhan Tang is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The survey was approved by National Center for Health Statistics (NCHS) Ethics Review Board.

Consent for publication

The authors have reviewed the manuscript and consent for publication.

Competing interests

No potential conflicts of interest relevant to this article were reported.

References

1. Rawshani A, Rawshani A, Franzen S, Sattar N, Eliasson B, Svensson AM, et al. Risk Factors, Mortality, and Cardiovascular Outcomes in Patients with Type 2 Diabetes. N Engl J Med. 2018;379(7):633–44.
2. Perreault L, Skyler JS, Rosenstock J. Novel therapies with precision mechanisms for type 2 diabetes mellitus. Nature Reviews Endocrinology. 2021;17(6):364–77.
3. Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. Nature Reviews Endocrinology. 2018;14(2):88–98.
4. Zimmet P, Alberti KG, Magliano DJ, Bennett PH. Diabetes mellitus statistics on prevalence and mortality: facts and fallacies. Nature Reviews Endocrinology. 2016;12(10):616–22.
5. Di Micco R, Krizhanovsky V, Baker D. d'Adda di Fagagna F. Cellular senescence in ageing: from mechanisms to therapeutic opportunities. Nature reviews Molecular cell biology. 2021;22(2):75–95.
6. Bellary S, Kyrou I, Brown JE, Bailey CJ. Type 2 diabetes mellitus in older adults: clinical considerations and management. Nature Reviews Endocrinology. 2021;17(9):534–48.

7. Huang ES, Laiteerapong N, Liu JY, John PM, Moffet HH, Karter AJ. Rates of Complications and Mortality in Older Patients With Diabetes Mellitus The Diabetes and Aging Study. Jama Internal Medicine. 2014;174(2):251–8.
8. Palmer AK, Gustafson B, Kirkland JL, Smith U. Cellular senescence: at the nexus between ageing and diabetes. Diabetologia. 2019;62(10):1835–41.
9. Hickson LJ, Prata LGPL, Bobart SA, Evans TK, Giorgadze N, Hashmi SK, et al. Senolytics decrease senescent cells in humans: Preliminary report from a clinical trial of Dasatinib plus Quercetin in individuals with diabetic kidney disease. Ebiomedicine. 2019;47:446–56.
10. Aguayo-Mazzucato C, Andle J, Lee T, Midha A, Talemal L, Chipashvili V, et al. Acceleration of β Cell Aging Determines Diabetes and Senolysis Improves Disease Outcomes. Cell Metabol. 2019;30(1):129–42.e4.
11. Cheng FF, Luk AO, Wu HJ, Lim CKP, Carroll L, Tam CHT, et al. Shortened relative leukocyte telomere length is associated with all-cause mortality in type 2 diabetes- analysis from the Hong Kong Diabetes Register. Diabetes Res Clin Pract. 2021;172:108649.
12. Astrup AS, Tarnow L, Jorsal A, Lajer M, Nzietchueng R, Benetos A, et al. Telomere length predicts all-cause mortality in patients with type 1 diabetes. Diabetologia. 2010;53(1):45–8.
13. Bonfigli AR, Spazzafumo L, Prattichizzo F, Bonafe M, Mensa E, Micolucci L, et al. Leukocyte telomere length and mortality risk in patients with type 2 diabetes. Oncotarget. 2016;7(32):50835–44.
14. Birch J, Anderson RK, Correia-Melo C, Jurk D, Hewitt G, Marques FM, et al. DNA damage response at telomeres contributes to lung aging and chronic obstructive pulmonary disease. American Journal of Physiology-Lung Cellular Molecular Physiology. 2015;309(10):L1124-L37.
15. Anderson R, Lagnado A, Maggiorani D, Walaszczyk A, Dookun E, Chapman J, et al. Length-independent telomere damage drives post-mitotic cardiomyocyte senescence. Embo Journal. 2019;38(5).
16. Liu Z, Kuo P, Horvath S, Crimmins E, Ferrucci L, Levine M. A new aging measure captures morbidity and mortality risk across diverse subpopulations from NHANES IV: A cohort study. PLoS Med. 2018;15(12):e1002718.
17. Han T, Gao J, Wang L, Li C, Qi L, Sun C, et al. The Association of Energy and Macronutrient Intake at Dinner Versus Breakfast With Disease-Specific and All-Cause Mortality Among People With Diabetes: The U.S. National Health and Nutrition Examination Survey, 2003-2014. Diabetes Care. 2020;43(7):1442–8.
18. Levine ME, Lu AT, Quach A, Chen BH, Assimes TL, Bandinelli S, et al. An epigenetic biomarker of aging for lifespan and healthspan. Aging-Us. 2018;10(4):573–91.
19. Klemera P, Doubal S. A new approach to the concept and computation of biological age. Mech Ageing Dev. 2006;127(3):240–8.
20. Levine M. Modeling the rate of senescence: can estimated biological age predict mortality more accurately than chronological age? The journals of gerontology Series A. Biological sciences. 2013;68(6):667–74.
21. Banach M, Mazidi M, Mikhailidis DP, Toth PP, Jozwiak J, Rysz J, et al. Association between phenotypic familial hypercholesterolaemia and telomere length in US adults: results from a multi-ethnic survey. Eur Heart J. 2018;39(40):3635–40.

22. Wang JF, Dong X, Cao L, Sun YY, Qiu Y, Zhang Y, et al. Association between telomere length and diabetes mellitus: A meta-analysis. J Int Med Res. 2016;44(6):1156–73.
23. Cheng FF, Carroll L, Joglekar MV, Januszewski AS, Wong KK, Hardikar AA, et al. Diabetes, metabolic disease, and telomere length. Lancet Diabetes Endocrinology. 2021;9(2):117–26.
24. Chen L, Mei GB, Jiang CJ, Cheng XE, Li D, Zhao Y, et al. Carbon monoxide alleviates senescence in diabetic nephropathy by improving autophagy. Cell Prolif. 2021;54(6):e13052.
25. Belsky D, Caspi A, Houts R, Cohen H, Corcoran D, Danese A, et al. Quantification of biological aging in young adults. Proc Natl Acad Sci USA. 2015;112(30):E4104-10.
26. Xu YC, Sun ZJ. Molecular Basis of Klotho: From Gene to Function in Aging. Endocr Rev. 2015;36(2):174–93.
27. Semba RD, Cappola AR, Sun K, Bandinelli S, Dalal M, Crasto C, et al. Plasma Klotho and Mortality Risk in Older Community-Dwelling Adults. Journals of Gerontology Series a-Biological Sciences and Medical Sciences. 2011;66(7):794–800.
28. Charoenngam N, Ponvilawan B, Ungprasert P. Lower circulating soluble Klotho level is associated with increased risk of all-cause mortality in chronic kidney disease patients: a systematic review and meta-analysis. Int Urol Nephrol. 2020;52(8):1543–50.
29. Torres PU, Prie D, Molina-Bletry V, Beck L, Silve C, Friedlander G. Klotho: An antiaging protein involved in mineral and vitamin D metabolism. Kidney Int. 2007;71(8):730–7.
30. Wan ZZ, Guo JY, Pan A, Chen C, Liu LG, Liu G. Association of Serum 25-Hydroxyvitamin D Concentrations With All-Cause and Cause-Specific Mortality Among Individuals With Diabetes. Diabetes Care. 2021;44(2):350–7.
31. Kulkarni AS, Gubbi S, Barzilai N. Benefits of Metformin in Attenuating the Hallmarks of Aging. Cell Metab. 2020;32(1):15–30.
32. Campbell JM, Bellman SM, Stephenson MD, Lisy K. Metformin reduces all-cause mortality and diseases of ageing independent of its effect on diabetes control: A systematic review and meta-analysis. Ageing Res Rev. 2017;40:31–44.
33. Barzilai N, Crandall JP, Kritchevsky SB, Espeland MA. Metformin as a Tool to Target Aging. Cell Metab. 2016;23(6):1060–5.
34. Cabreiro F, Au C, Leung KY, Vergara-Irigaray N, Cocheme HM, Noori T, et al. Metformin Retards Aging in C. elegans by Altering Microbial Folate and Methionine Metabolism. Cell. 2013;153(1):228–39.
35. Yang CB, Zhang W, Dong XD, Fu CJ, Yuan JM, Xu ML, et al. A natural product solution to aging and aging-associated diseases. Pharmacol Ther. 2020;216:107673.
36. Novais EJ, Tran VA, Johnston SN, Darris KR, Roupas AJ, Sessions GA, et al. Long-term treatment with senolytic drugs Dasatinib and Quercetin ameliorates age-dependent intervertebral disc degeneration in mice. Nat Commun. 2021;12(1):5213.
37. Justice J, Nambiar A, Tchkonia T, LeBrasseur N, Pascual R, Hashmi S, et al. Senolytics in idiopathic pulmonary fibrosis: Results from a first-in-human, open-label, pilot study. EBioMedicine. 2019;40:554–63.

Figures

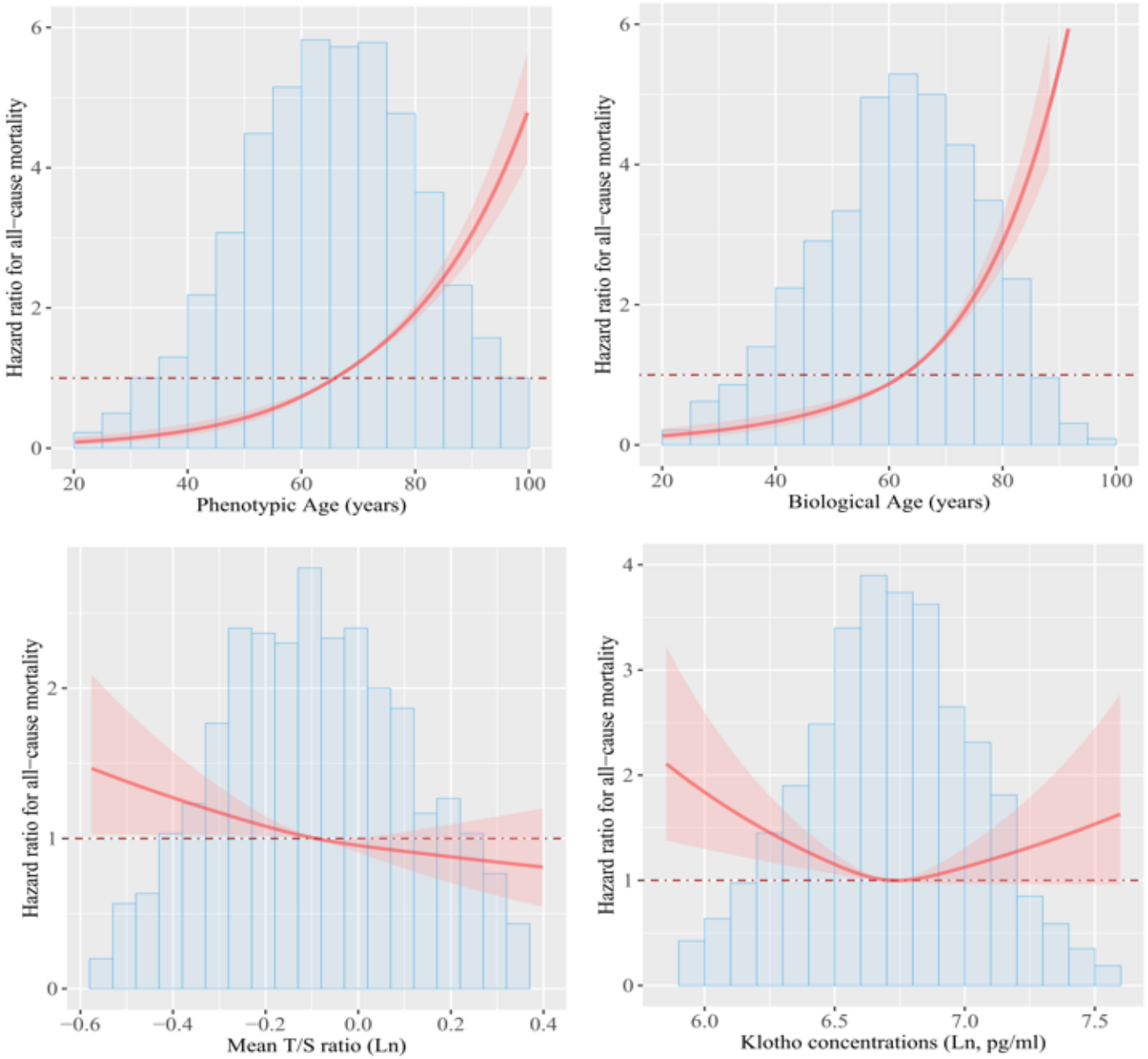

Figure 1

The dose-response relationship between aging markers and all-cause mortality assessed by restricted cubic spline regression. Models were adjusted for age (<65 or ≥65), sex (male or female), race/ethnicity (Mexican American, other Hispanic, non-Hispanic White, non-Hispanic Black, or other), marital status (married/cohabiting, widowed/divorced/separated, or never married), BMI (<25.0, 25.0-29.9, or ≥30.0 kg/m2), physical activity (moderate or vigorous), drinking alcohol status (ever or never), Ln-cotinine concentration (continuous), triglyceride (continuous), medication use (insulin/pills or no), CVD (yes or no), cancer (yes or no), and hypertension (yes or no).

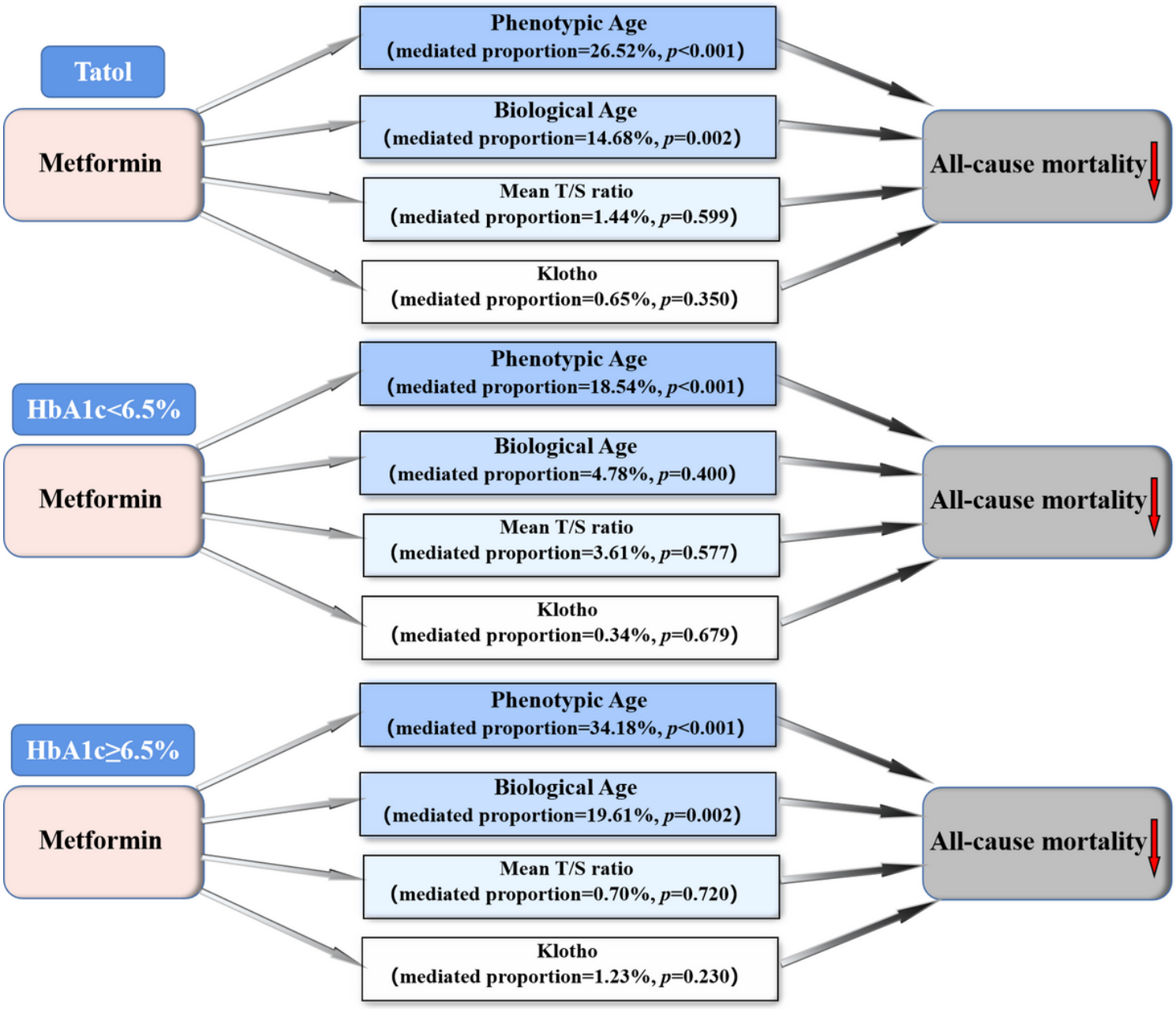

Figure 2

The mediation effects of aging markers on associations of metformin use with all-cause mortality stratified by HbA1c level. Models were adjusted for age (<65 or ≥65 years), sex (male or female), race/ethnicity (Mexican American, other Hispanic, non-Hispanic White, non-Hispanic Black, or other), marital status (married/cohabiting, widowed/divorced/separated, or never married), BMI (<25.0, 25.0-29.9, or ≥30.0 kg/m2), physical activity (moderate or vigorous), drinking alcohol status (ever or never), Ln-cotinine concentration (continuous), triglyceride (continuous), medication use (insulin/pills or no), CVD (yes or no), cancer (yes or no), and hypertension (yes or no).

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