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Mitochondria-derived methylmalonic acid, a surrogate biomarker of mitochondrial dysfunction and oxidative stress, predicts all-cause and cardiovascular mortality in the general population

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ABSTRACT

Background: Inherited methylmalonic acidemia is characterized by mitochondrial dysfunction, oxidative stress, and damage of mitochondria-rich organs in children. It is unclear whether methylmalonic acid (MMA) is related to poor prognosis in adults. The study aims to investigate the associations of MMA with all-cause and cause-specific mortality in the general population.

Methods: Overall, 23,437 adults from the US National Health and Nutrition Examination Survey (NHANES) were enrolled. NHANES 1999–2004 and 2011–2014 were separately used as primary and validation subsets (median follow-up 13.5 and 2.8 years, respectively). Circulating MMA was measured with gas chromatography/mass spectrophotometry. Hazard ratios (HR) were estimated using weighted Cox regression models.

Results: During 163,632 person-years of follow-up in NHANES 1999–2004, 3019 deaths occurred. Compared with participants with MMA <120 nmol/L, those with MMA≥250 nmol/L had increased all-cause and cardiovascular mortality in the multivariable-adjusted model [HR(95%CI), 1.62 (1.43–1.84) and 1.66 (1.22–2.27), respectively]. The association was especially significant among participants with normal cobalamin. MMA remained an independent predictor of all-cause mortality occurring whether within 5-year, 5–10 years, or beyond 10-year of follow-up (each p for trend≤0.007). That association was repeatable in NHANES 2011–2014. Moreover, baseline MMA improved reclassification for 10-year mortality in patients with cardiovascular disease (net reclassification index 0.239, integrated discrimination improvement 0.022), overmatched established cardiovascular biomarkers C-reactive protein or homocysteine.

Conclusions: Circulating level of mitochondrial-derived MMA is strongly associated with elevated all-cause and cardiovascular mortality. Our results support MMA as a surrogate biomarker of mitochondrial dysfunction to predict poor prognosis in adults. The biological mechanisms under cardiovascular disease warrant further investigation.

Introduction

Rapidly increasing morbidity and mortality of chronic disease remain one of the leading health challenges in the 21st century worldwide [1]. Mitochondrial impairment and redox imbalance have been implicated in the pathogenesis of various chronic diseases, such as coronary heart disease, heart failure, dementia, and diabetes [2–5]. Our previous experimental studies suggested improving mitochondrial injury and oxidative stress via genetic and pharmaceutical treatments could alleviate the severity of diabetic cardiomyopathy and myocardial infarction in mice model [6–9]. Mitochondria have been considered as

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Abbreviations

AIC	Akaike information criterion
ALT	Alanine aminotransferase
BIC	Bayesian information criterion
BMI	Body mass index
BP	Blood pressure
Cbl, B12	Cobalamin, Vitamin B12
CLRD	Chronic lower respiratory diseases
COPD	Chronic obstructive pulmonary disease
CRP	C-reactive protein
CV	Coefficient of variation
CVD	Cardiovascular disease
eGFR	Estimated glomerular filtration rate
Hcy	Homocysteine
HDL-C	High-density lipoprotein cholesterol
IDI	Integrated discrimination improvement
MEC	Mobile examination centers
MMA	Methylmalonic acid
MUT	Methylmalonyl-CoA mutase
NRI	Net reclassification improvement
ROS	Reactive oxygen species

the key regulatory center uniquely located at the crossroads of multiple cellular processes. Targeting mitochondrial processes seem one of the most promising approaches to identify novel disease biomarker and therapeutic strategy [3,7,10].

Methylmalonic acid (MMA), a mitochondrial intermediate metabolite of odd-chain fatty acids and several amino acids (isoleucine, valine, methionine, and threonine) catabolism, is converted into succinic acid and enters the Krebs cycle under normal conditions [11,12]. The metabolism of MMA relies on healthy mitochondria. This process can be hindered by mitochondrial methylmalonyl-CoA mutase (MUT) deactivation or coenzyme active Vitamin B12 (cobalamin, Cbl) deficiency, leading to MMA accumulation [11]. It is noteworthy that, increasing findings evidence a key role of MMA in mitochondrial dysfunction and oxidative stress both in vitro and in vivo, partly by disturbing the mitochondrial respiratory chain and inducing reactive oxygen species (ROS) generation [13,14]. Inborn methylmalonic acidemia causes serious injury in mitochondria-rich organs, including cerebropathy nephropathy and cardiomyopathy in children [13,15,16]. Blood MMA may be a favorable candidate marker of mitochondrial dysfunction and oxidative stress to predict adverse outcomes for chronic disease [17]. Currently, circulating MMA has been used to screen genetic methylmalonic acidemia and Cbl deficiency in clinic [11,18]. The prognostic value of MMA has not been reported in adults. To extend the clinical understanding of postnatal MMA increase, the study determines the association between circulating MMA and all-cause or cause-specific mortality in the general population.

Methods

Study population

All data were extracted from the National Health and Nutrition Examination Surveys (NHANES) conducted by the Center for Disease Control and Prevention of the United States. As described in our previous reports [19], NHANES is an ongoing, national, stratified, and multistage probability sample to investigate the health status of the non-institutionalized civilian population of all ages. In the current study, 26,661 participants 20 years or older in five 2-year NHANES cycles (1999–2000, 2001–2002, 2003–2004, 2011–2012, and 2013–2014) were included. We excluded individuals without eligible MMA data (n = 3192) and loss of follow-up for mortality status (n = 32). Lastly, 23,437 people were available for analysis (Fig. S1). The first 3 cycles NHANES 1999–2004 were combined as a subset for primary analysis (n = 13,259) and the latter two were combined to validate the relationship between MMA and mortality risk (n = 10,178). The research protocols were approved by the ethics review board of the National Center for Health Statistics. All participants provided informed consent. Information in detail is available on the website http://www.cdc.gov/nchs/nhanes/irba98.htm.

Circulating MMA measurement

Blood was collected via venepuncture in mobile examination centers (MEC) according to the standardized protocols. MMA was determined in plasma and/or serum, preferentially in plasma. Prior studies confirmed MMA concentrations in serum and plasma having a common reference range and similar coefficient of variation (CV) [20,21]. The detection protocol has been described in detail on the website of NHANES. Due to both isomers with the same molecular weight, it had been validated that MMA could be distinguished from succinic acid through chromatographic separation. The measurement of MMA was conducted utilizing gas chromatography/mass spectrophotometry. In brief, 275 μL specimens with added internal standard solution supplemented by isotope-labeled methyl-d^3-malonic acid (d^3MMA) were extracted and subsequently derivatized with cyclohexanol to produce a dicyclohexyl ester. After the procedures of derivatization and separation, the effluent part from the gas chromatograph was monitored with a mass selective detector by the selected ion monitoring process. MMA level was quantitated by the peak area ratios of MMA and isotope-labeled d^3MMA. There was a favorable linear pattern for MMA in the range of 50–2000 nmol/L. Samples with MMA concentrations >400 nmol/L were repeatedly analyzed. The total CV was 4–10% and the mean recovery rate was 96.0 ± 1.9%, as described in NHANES laboratory protocols.

Definition of covariates

General information on age, sex, race/ethnicity, family income, smoking status, physical activity, and drink was collected using standardized questionnaires during interviews [22]. Race/ethnicity was categorized as non-Hispanic white, non-Hispanic black, Hispanic-Mexican and the other. Smoking status was classified as never, ever, and current smoking. Ever smokers were defined as participants who ever smoked at least 100 cigarettes but quitting now. Leisure physical activity during the past month was classified as vigorous, moderate, and less exercise as our previous described [19].

Physical examinations were conducted in MEC. Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared. Average systolic blood pressure (BP) or diastolic BP was estimated as the mean of eligible three readings [22]. Laboratory examination was performed under fasting or non-fasting conditions following the standardized procedures. Samples with fasting status were defined as the time of last ate or drank anything other than water until venipuncture ≥8 h. Laboratory data on triglyceride (TG), total cholesterol (TC), High-density lipoprotein-cholesterol (HDL-C), HbA1c, insulin, alanine aminotransferase (ALT), creatinine, MMA, and Cbl were measured in all cycles, but C-reactive protein (CRP) and Hcy were just determined in NHANES 1999–2004. The estimated glomerular filtration rate (eGFR) was calculated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI). Diabetes mellitus was identified by anti-hyperglycemic therapy or HbA1c ≥ 6.5% [22]. Hypertension was defined as antihypertensive medication, the average systolic BP ≥ 140 mmHg, or diastolic BP ≥ 90 mmHg at baseline. Cardiovascular disease (CVD) was defined as a self-reported history of coronary heart disease, heart failure, or stroke according to previous NHANES reports [22]. Chronic obstructive pulmonary disease (COPD) was defined as emphysema or chronic bronchitis. Malignancy was self-reported.

Death ascertainment

All participants aged 18 years or older in NHANES were linked to the National Death Index with a unique sequence number until December 31, 2015 [19]. The leading cause of death was ascertained according to the codes of International Classification of Diseases 10th Revision (ICD-10). We assessed all-cause mortality and mortality due to CVD (heart disease: I00–I09, I11, I13, I20–I51; and cerebrovascular disease: I60–I69), cancer (C00–C97), chronic lower respiratory diseases (CLRD, J40-J47), Alzheimer's disease (G30), diabetes mellitus (E10-E14), and kidney disease (N00–N07, N17–N19, N25–N27). The primary endpoint was defined as all-cause and cardiovascular mortality.

Statistical analysis

Statistical analysis was conducted with Stata (version 15.0) and R (version 3.6). All estimates were weighted with masked variance of primary sampling unit, pseudo-strata and appropriate sampling weights to account for the complex sampling design unless otherwise noted. Continuous and categorical variables are expressed as weighted mean (SE) and percentage. Partial correlation analysis adjusted for age, sex, and race between the natural logarithm of MMA and cardiometabolic markers was conducted using Pearson correlation.

Restricted cubic spline with age-, gender- and race-adjusted Cox regression model was used to visualize the relationship between log-transformed MMA and all-cause mortality. We found that all-cause mortality was not significantly increased when MMA<120 nmo/L (Fig. 1). There lacked a unified standard to stratify MMA levels, and prior studies defined plasma MMA ≥250 nmol/L as one of the diagnostic criteria for functional VitB12 deficiency [23]. Thus, participants were categorized as four groups according to MMA level: <120 nmol/L, 120–175 nmol/L, 175–250 nmol/L, and ≥250 nmol/L. Trend analysis of baseline characteristics across MMA strata was evaluated by linear regression for continuous variables and logistic regression for categorical variables after weighting.

The Poisson distribution was used to estimate mortality rates and 95% CI, presented as mortality per 1000 person-years of follow-up. Crude and multivariable Cox proportional hazards regression were applied to assess the association of MMA with all-cause and cause-specific mortality. Two adjusted models were adopted. Model 1 was adjusted for age (years, continuous), sex (female or male), and race/ethnicity (non-Hispanic white, black, Hispanic-Mexican, or other). Model 2 was additionally adjusted for poverty to income ratio (<1.3, 1.3–3.5, ≥3.5, or missing), body mass index (<18.5, 18.5–25, 25–30, or ≥30 kg/m2), smoking status (never, ever or current), alcohol consumption (no, <5, 5–30, ≥30 g/d, or missing), physical activity (inactive, moderate, or vigorous), hypertension (no/yes), fasting status (no/yes), total cholesterol (mmol/L, continuous), HDL-C (mmol/L, continuous), CRP (mg/dL, continuous, only for NHANES 1999–2004), alanine aminotransferase (U/L, continuous), estimated glomerular filtration rate (mL/min/1.73 m^2, continuous), CVD (no/yes), diabetes (no/yes), COPD (no/yes), cancer (no/yes), serum homocysteine (μmol/L, continuous, only for NHANES 1999–2004), cobalamin (pmol/L, continuous) and metformin use (no/yes).

We further applied stratification analysis for the association between baseline MMA and all-cause mortality in subgroups by age (<65 and ≥ 65 years), sex (female and male), BMI (<30 and ≥ 30 kg/m2), current smoker (no/yes), eGFR (≥60 and < 60 mL/min per 1.73 m^2), Hcy (<10 and ≥ 10 μmol/L), Vit B12 (<400 and ≥ 400 pmol/L), fasting status(no/yes), and the number of cardiovascular risk factors (≤1 and ≥ 2). The Survey-weighted Wald test was used to evaluate the potential interaction between MMA and stratification factors for mortality.

Additional analysis regarding the association of MMA and all-cause mortality during 5-year bands (0–5, 5–10, and more than 10 years) was carried out to determine whether the relationship varied over time.

The robustness of the association between MMA and all-cause mortality was further validated in NHANES 2011–2014 with adjustment for the aforementioned potential confounders except for CRP and Hcy.

The predictive value of MMA in mortality risk was assessed in CVD patients of NHANES 1999–2004 and 2010–2014 separately. The reference model was derived from the Framingham risk score, including age, sex, Charlson comorbidity index, systolic BP, TC, and HDL-C [24]. Goodness of Fit was assessed using the likelihood ratio test, Akaike information criterion (AIC), and Bayesian information criterion (BIC). Harrell's C-index, net reclassification improvement (NRI), and integrated discrimination improvement (IDI) were used to assess the ability of discrimination. All tests with a 2-sided $P < 0.05$ were considered statistically significant unless otherwise noted.

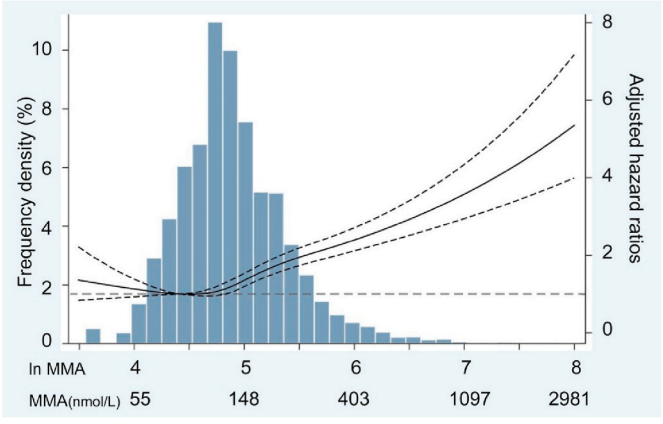

Fig. 1. The distribution of MMA and its adjusted hazard ratio (HR) for all-cause mortality in NHANES 1999–2004. The histogram represents the distribution of circulating MMA level in the population (left Y-axis). The restricted cubic spline curve shows the adjusted hazard ratios of log-transformed MMA for all-cause mortality based on Cox regression model (right Y-axis, treating MMA level = 120 nmol/L as the reference). Knots include the 5th, 27.5th, 50th, 72.5th, and 95th percentiles of the natural logarithm of MMA. The model was adjusted for sex, age, and race/ethnicity. The solid line represents point estimates, and dashed lines represent 95% CIs. The association of log MMA with mortality was J-shaped. Participants with MMA more than 120 nmol/L may have an increased risk of all-cause mortality.

Results

Participant characteristics

Baseline characteristics across MMA strata of the 13,259 participants in NHANES 1999–2004 were shown in Table 1. Overall, the mean age was 46.2 years and 52% were female. Participants with higher MMA levels were more likely to be older and have a higher proportion of non-black. They were also more often taking less exercise, suffering from comorbidities, and having higher levels of cardiac risk factors, including systolic BP, CRP, TC, Hcy, and creatinine. Baseline characteristics in the validation subset NHANES 2011–2014 were comparable to NHANES 1999–2004 except for serum Hcy and CRP (Table S1). Geometric mean of blood MMA at baseline was 136.5 nmol/L and their concentrations were less variable from 1999-2000 cycle to 2013–2014 cycle (Fig. 1 and S2). There was a minor difference in MMA level between nonfasting and fasting status (Fig. S3).

Correlation of MMA with cardiometabolic biomarkers

Partial correlation coefficients adjusted for age, gender, and race were shown in Table S2. Circulating MMA was positively correlated with serum creatinine and Hcy (r = 0.25 and 0.35, respectively), as well as inversely correlated with serum Cbl (r = -0.3). Whereas, the correlation with blood pressure, BMI, glucose, HbAc1, insulin resistance index, lipid

Table 1
Baseline characteristics of participants in NHANES 1999–2004 by baseline methylmalonic acid levels.

Variables	Circulating MMA, nmol/L				p trend
	<120 (n = 5337)	120-175 (n = 4580)	175-250 (n = 1969)	≥250 (n = 1373)	
Age,y	41.1 ± 0.34	46.9 ± 0.32	51.3 ± 0.42	57.1 ± 0.74	<0.001
Sex, %					0.015
Female	55.5	49.6	48.4	53.1	
Male	44.5	50.4	51.6	47.0	
Race/ethnicity, %					
Non-Hispanic White	61.6	77.1	81.6	77.8	<0.001
Non-Hispanic Black	15.4	8.3	5.8	5.8	<0.001
Hispanic-Mexican	10.8	5.5	4.2	4.4	<0.001
Other Ethnicity	12.2	9.1	8.4	12.1	0.298
Poverty to income ratio	2.9 ± 0.05	3.1 ± 0.05	3.0 ± 0.06	2.6 ± 0.07	<0.001
BMI, kg/m^2	28.1 ± 0.14	28.1 ± 0.13	28.3 ± 0.17	27.8 ± 0.24	0.627
Smoking status					0.006
Never smoking	52.4	49.9	47.9	45.0	
Former smoker	22.3	26.6	26.6	31.3	
Current smoker	25.3	23.5	25.4	23.8	
Alcohol consumption, g/day	4.7 ± 0.23	5.0 ± 0.24	5.4 ± 0.41	4.0 ± 0.38	0.336
Physical activity, %					<0.001
Inactive	35.0	34.6	41.2	52.4	
Moderate activity	27.9	29.7	30.8	29.3	
Vigorous activity	37.2	35.6	28.0	18.4	
Hypertension, %	27.9	35.5	43.5	51.5	<0.001
Fasting status, %	70.6	64.5	61.5	59.0	<0.001
Total cholesterol, mmol/L	5.1 ± 0.02	5.2 ± 0.03	5.2 ± 0.03	5.2 ± 0.04	0.131
HDL-C, mmol/L	1.4 ± 0.01	1.3 ± 0.01	1.3 ± 0.01	1.3 ± 0.02	0.047
C-reactive protein, mg/dL	0.4 ± 0.01	0.4 ± 0.01	0.5 ± 0.02	0.5 ± 0.03	0.103
ALT, U/L	26.6 ± 0.39	26.1 ± 0.35	25.4 ± 0.54	24.5 ± 2.43	0.283
eGFR, mL/min per 1.73m^2	107.2 ± 0.58	97.1 ± 0.45	89.4 ± 0.56	80.8 ± 1.07	<0.001
Cardiovascular disease, %	4.9	7.5	13.4	23.4	<0.001
Diabetes, %	6.3	7.0	9.9	14.8	<0.001
COPD, %	6.4	7.7	9.0	11.6	<0.001
Cancer, %	5.2	8.1	11.1	14.3	<0.001
Homocysteine, μmol/L	7.6 ± 0.06	8.6 ± 0.07	9.8 ± 0.11	12.8 ± 0.32	<0.001
Vitamin B12, pmol/L	457.9 ± 24.65	374.8 ± 3.35	348.2 ± 11.89	312.9 ± 16.71	<0.001
Metformin, %	2.4	2.8	3.4	2.6	0.201
Number of CV risk factor*					<0.001
None	36.3	30.0	24.7	20.3	
1	36.6	38.4	35.3	33.7	
2	21.0	24.8	29.3	33.9	
≥3	6.1	6.8	10.7	12.1	

Variables are presented as weighted proportion (%) or mean ± SE. P for trend was estimated with linear regression for continuous variables and with logistic regression for categorical variables. *CV risk factor included current smoking, hypertension, dyslipidemia, and diabetes.
BMI, body mass index; BP, blood pressure; COPD, chronic obstructive pulmonary disease; CV, cardiovascular; eGFR, estimated glomerular filtration rate; ALT, alanine aminotransferase; HDL-C, high-density lipoprotein cholesterol.
Variables with missing value included poverty to income ratio (n = 1124), BMI (n = 385), smoking (n = 21), alcohol use (n = 867), hypertension (n = 9), fasting status (n = 10), TC (n = 202), HDL-C (n = 187), C-reactive protein (n = 114), ALT (n = 207), eGFR (n = 204), Cardiovascular disease (n = 2), COPD (n = 3), cancer(n = 19), Homocysteine (n = 8), and vitamin B12 (n = 115).

metabolism, CRP, and liver function was insignificant or weak. Thus, MMA might serve as a potential biomarker distinctive from traditional risk factors. Since 34.5% had laboratory blood tests under non-fasting status, the correlation analysis was further restricted to participants with fasting time ≥ 8 h. The associations remained unchanged.

Association of MMA with mortality in NHANES 1999–2004

Over a median follow-up of 13.5 (interquartile range, 11.8–15.0) years, 3019 individuals died, including 708 CVD and 628 cancer deaths. The weighted mortality was presented as a rate per 1000 person-years of follow-up, with 12.2 (95% CI, 11.6–12.8) for all-cause, 2.6 (95% CI, 2.4–2.9) for CVD, and 2.7 (95% CI, 2.5–3.0) for cancer mortality. There was a significant dose-response trend across MMA strata for all-cause and cause-specific mortality (Fig. 2 and Table S2).

The associations of MMA with all-cause and cause-specific mortality were further investigated by weighted Cox regression analysis. Both in crude and age-, sex- and race/ethnicity-adjusted models, participants with higher MMA concentration had an increased risk of all-cause mortality (Fig. 1 and Table 2). After fully adjusting for 22 covariates, the association still complied with a dose-response pattern, with an elevated risk of all-cause mortality by 33% per each unit increase of log-transformed MMA (HR 1.33 95%CI 1.21–1.47, p < 0.001). When analyzed categorical MMA, the linear relationship remained significant in the fully adjusted model (p for trend <0.001). Compared with those with MMA<120 nmol/L, the HRs (95%CI) of all-cause mortality in participants with MMA 120–175 nmol/L, 175–250 nmol/L, and >250 nmol/L were 1.11 (0.98–1.26), 1.37 (1.18–1.60) and 1.62 (1.43–1.84).

As expected, baseline MMA levels were robustly associated with higher mortality due to CVD, cancer, CLRD, Alzheimer's disease, diabetes mellitus, and nephropathy in univariate analysis (Table 2 and Table S4). In age-, gender- and race-adjusted model, MMA level was not correlated with CLRD- and dementias-related deaths, while participants with higher MMA had a significant elevation of mortality due to CVD, cancer, diabetes mellitus, and kidney disease. After full adjustment for potential covariates, only cardiovascular mortality remained significantly increased across MMA strata, especially the heart-related mortality. Compared with participants with MMA <120 nmol/L, there was a 67% increased risk of heart-related mortality among those having MMA> 250 nmol/L (HR 1.67, 95%CI 1.19–2.34). However, the association between MMA level and cancer mortality was attenuated and became marginally significant.

Restricted to healthy persons who were free from CVD and cancer at baseline, the relationship between elevated MMA levels and all-cause and cardiovascular mortality was relatively attenuated but remained significant (Table S5). According to weighted Kaplan-Meier curves (Fig. 2), even beyond 10 years of follow-up, all-cause mortality across MMA strata continued to diverge well over time. Consistently, according to prespecified Cox regression, an increase of MMA level at baseline was significantly associated with higher all-cause mortality for those events occurring within 5 years, 5–10 years or beyond 10 years of follow-up (Table 3).

In stratification analyses (Table 4), the dose-response relationship between MMA and elevated all-cause mortality was largely consistent in subgroups by age (<65 and ≥ 65 years), sex, BMI (<30 and ≥ 30 kg/m2), current smoker, chronic kidney disease, serum Hcy (<10 and ≥ 10 μmol/L), fasting status and the number of cardiovascular risk factors (all p ≥ 0.212 for interaction). However, a significant interaction on all-cause mortality was noted between MMA and Vitamin B12 (p < 0.001 for interaction). The HRs(95%CIs) per each unit increase of log-transformed MMA for all-cause deaths were 1.25 (1.13–1.38) among participants with B12 < 400 pmol/L versus 1.69 (1.35–2.12) among participants with B12 ≥ 400 pmol/L.

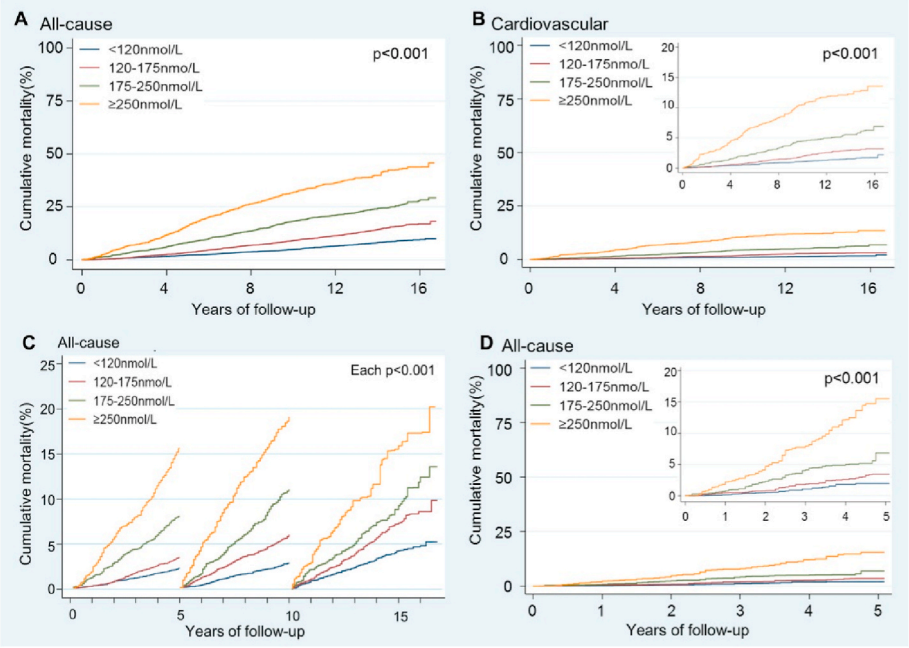

Fig. 2. Kaplan-Meier plots for all-cause and cardiovascular mortality by baseline methylmalonic acid strata. **A and B,** All-cause and cardiovascular mortality by MMA groups in NHANES 1999–2004; **C,** All-cause mortality within each segmented band, including 5-year, during 5–10 years, or beyond 10-year of follow-up in NHANES 1999–2004; **D,** All-cause mortality in NHANES 2011–2014. Y-axis shows the absolute risk of cumulative mortality rate (%) estimated by survey-weighted Kaplan-Meier failure function. X-axis shows the follow-up period (years). The curves presented in the upper right corner of B and D show the same data on a truncated Y-axis. Participants with higher MMA levels had increased risks of all-cause and cardiovascular mortality.

Table 2
The association of methylmalonic acid with all-cause mortality, cardiovascular and cancer mortality in NHANES 1999–2004.

	MMA, nmol/L						p trend
Cause of death	log MMA[a] (n = 13,259)	p value	<120 (n = 5337)	120-175 (n = 4580)	175-250 (n = 1969)	≥250 (n = 1373)	
All-cause mortality							
Deaths/person-yrs	3019/163632	–	591/70541	957/57739	718/22133	753/13219	–
Crude	2.78 (2.56–3.02)[b]	<0.001	1 (ref.)	1.82 (1.61–2.07)	3.36 (2.89–3.91)	6.36 (5.66–7.16)	<0.001
Model 1	1.62 (1.50–1.75)	<0.001	1 (ref.)	1.13 (1.01–1.26)	1.57 (1.36–1.81)	2.16 (1.89–2.47)	<0.001
Model 2	1.37 (1.24–1.51)	<0.001	1 (ref.)	1.11 (0.98–1.26)	1.37 (1.18–1.60)	1.62 (1.43–1.84)	<0.001
CVD mortality							
Deaths/person-yrs	708/163632	–	130/70541	208/57739	165/22133	205/13219	–
Crude	3.11 (2.77–3.50)	<0.001	1 (ref.)	1.85 (1.39–2.47)	3.71 (2.78–4.96)	8.79 (6.81–11.33)	<0.001
Model 1	1.81 (1.57–2.09)	<0.001	1 (ref.)	1.06 (0.81–1.38)	1.50 (1.18–1.91)	2.48 (1.91–3.23)	<0.001
Model 2	1.42 (1.15–1.76)	0.001	1 (ref.)	1.05 (0.78–1.42)	1.25 (0.94–1.66)	1.66 (1.22–2.27)	<0.001
Heart mortality							
Deaths/person-yrs	532/163632	–	98/70541	145/57739	132/22133	157/13219	–
Crude	3.18 (2.78–3.65)	<0.001	1 (ref.)	1.63 (1.21–2.19)	3.58(2.65–4.84)	8.73 (6.56–11.6)	<0.001
Model 1	1.89 (1.60–2.23)	<0.001	1 (ref.)	0.93 (0.70–1.25)	1.47(1.13–1.91)	2.52 (1.86–3.41)	<0.001
Model 2	1.47 (1.18–1.83)	0.001	1 (ref.)	0.94 (0.67–1.31)	1.28 (0.94–1.74)	1.67 (1.19–2.34)	<0.001
Stroke mortality							
Deaths/person-yrs	176/163632	–	32/70541	63/57739	33/22133	48/13219	–
Crude	2.87 (2.32–3.55)	<0.001	1 (ref.)	2.81 (1.54–5.12)	4.28 (2.38–7.69)	9.05 (4.52–18.13)	<0.001
Model 1	1.54 (1.10–2.15)	0.013	1 (ref.)	1.57 (0.88–2.81)	1.66 (0.92–2.98)	2.38 (1.19–4.76)	0.025
Model 2	1.24 (0.79–1.95)	0.340	1 (ref.)	1.49 (0.84–2.65)	1.12 (0.65–1.93)	1.61 (0.80–3.25)	0.376
Cancer mortality							
Deaths/person-yrs	628/163632	–	138/70541	223/57739	148/22133	119/13219	–
Crude	2.24 (1.91–2.61)	<0.001	1 (ref.)	1.87 (1.43–2.44)	2.89 (1.98–4.22)	4.41 (3.13–6.21)	<0.001
Model 1	1.28 (1.03–1.60)	0.030	1 (ref.)	1.23 (0.94–1.59)	1.47 (1.00–2.17)	1.70 (1.16–2.50)	0.008
Model 2	1.22 (0.93–1.58)	0.142	1 (ref.)	1.18 (0.92–1.53)	1.39 (0.97–1.97)	1.58 (1.07–2.32)	0.023

CVD, cardiovascular disease; Ref, treating the bottom group (MMA<120 nmol/L) as the reference.

[a] Hazard ratio per 1 unit increase of natural log-transformed MMA.

[b] Values are weighted hazard ratio (95% confidence interval). Model 1 (n= 13,259): adjusted for age (years, continuous), sex (female or male), and race/ethnicity (non-Hispanic white, black, Hispanic-Mexican, or other). Mode 2 (n= 12,940): additionally adjusted for poverty to income ratio (<1.3, 1.3-3.5, ≥3.5, or missing), body mass index (<18.5, 18.5–25, 25–30, or ≥30 kg/m2), smoking status (never, ever or current), alcohol consumption (no, <5, 5-30, ≥30g/d, or missing), physical activity (inactive, moderate, or vigorous), hypertension (no/yes), fasting status (no/yes), total cholesterol (mmol/L, continuous), high-density lipoprotein cholesterol (mmol/L, continuous), C-reactive protein (mg/dL, continuous), alanine aminotransferase (U/L, continuous), estimated glomerular filtration rate (mL/min/1.73m^2, continuous), cardiovascular diseases (no/yes), diabetes (no/yes), chronic obstructive pulmonary disease (no/yes), cancer (no/yes), serum homocysteine (μmol/L, continuous), cobalamin (pmol/L, continuous) and metformin use (no/yes).

Validation of the association in NHANES 2011–2014

The relationship between MMA and all-cause, heart-related, and cancer mortality was validated in 10,178 participants of NHANES 2011–2014. During a median follow-up of 2.8 years (29,477 person-years), 342 individuals died, with a weighted incidence rate of 9.2 per 1000 person-years. According to the Kaplan-Meier curve and multivariable Cox regression analysis, the association of MMA with mortality risk

Table 3
Associations between methylmalonic acid at baseline and all-cause mortality within 5-year periods in NHANES 1999–2004.

	Circulating MMA (nmol/L)						p trend
	log MMA[a]	p value	<120 (n = 5337)	120-175 (n = 4580)	175-250 (n = 1969)	≥250 (n = 1373)	
Within 5 years	n = 13259						
Crude	2.80 (2.49–3.14)[b]	<0.001	1 (ref.)	1.57 (1.21–2.02)	3.74 (2.93–4.77)	7.61 (5.94–9.75)	<0.001
Model 1	1.71 (1.53–1.90)	<0.001	1 (ref.)	0.99 (0.77–1.27)	1.70 (1.31–2.20)	2.39 (1.81–3.17)	<0.001
Model 2	1.26 (1.05–1.52)	0.015	1 (ref.)	0.96 (0.72–1.26)	1.42 (1.07–1.89)	1.57 (1.16–2.14)	<0.001
5–10 years	n = 12233						
Crude	2.94 (2.61–3.32)	<0.001	1 (ref.)	2.17 (1.68–2.81)	4.15 (3.10–5.54)	7.64 (5.74–10.18)	<0.001
Model 1	1.69 (1.49–1.91)	<0.001	1 (ref.)	1.30 (1.01–1.68)	1.86 (1.41–2.47)	2.46 (1.85–3.28)	<0.001
Model 2	1.51 (1.28–1.79)	<0.001	1 (ref.)	1.30 (0.98–1.72)	1.67 (1.22–2.27)	1.94 (1.41–2.68)	<0.001
Beyond 10 years	n = 11070						
Crude	2.45 (2.18–2.75)	<0.001	1 (ref.)	1.72 (1.34–2.20)	2.34 (1.78–3.07)	3.97 (3.14–5.02)	<0.001
Model 1	1.42 (1.22–1.65)	<0.001	1 (ref.)	1.07 (0.81–1.40)	1.19 (0.90–1.59)	1.69 (1.28–2.22)	<0.001
Model 2	1.28 (1.09–1.52)	0.004	1 (ref.)	1.06 (0.82–1.36)	1.04 (0.78–1.39)	1.40 (1.12–1.75)	0.007

Ref, treating the bottom group (MMA<120 nmol/L) as reference.

[a] Hazard ratio per 1 unit increase of natural log-transformed MMA.

[b] Values are hazard ratio (95% confidence interval). Model 1: adjusted for age (years, continuous), sex (female or male), and race/ethnicity (non-Hispanic white, black, Hispanic-Mexican, or other).Model 2: additionally adjusted for poverty to income ratio (<1.3, 1.3-3.5, ≥3.5, or missing), body mass index (<18.5, 18.5–25, 25–30, or ≥30 kg/m2), smoking status (never, ever or current), alcohol consumption (no, <5, 5-30, ≥30g/d, or missing), physical activity (inactive, moderate, or vigorous), hypertension (no/yes), fasting status (no/yes), total cholesterol (mmol/L, continuous), high-density lipoprotein cholesterol (mmol/L, continuous), C-reactive protein (mg/dL, continuous), alanine aminotransferase (U/L, continuous), estimated glomerular filtration rate (mL/min/1.73m^2, continuous), cardiovascular diseases (no/yes), diabetes (no/yes), chronic obstructive pulmonary disease (no/yes), cancer (no/yes), serum homocysteine (μmol/L, continuous), cobalamin (pmol/L, continuous) and metformin use (no/yes).

Table 4
Stratification analysis for the HRs of all-cause mortality per one unit increase of log-transformed MMA or across MMA strata in NHANES 1999–2004.

	Circulating MMA (nmol/L)							p for interaction<
Subgroup	log MMA	p value	<120	120–175	175–250	≥250	p trend	
Age, years								0.825
<65	1.36 (1.17–1.58)[a]	<0.001	1 (ref.)	1.04 (0.82–1.33)	1.19 (0.95–1.49)	1.51 (1.14–2.00)	0.003	
≥65	1.38 (1.22–1.57)	<0.001	1 (ref.)	1.36 (1.15–1.60)	1.76 (1.48–2.09)	1.98 (1.64–2.39)	<0.001	
Sex								0.212
Female	1.35 (1.15–1.60)	0.001	1 (ref.)	1.13 (0.93–1.38)	1.58 (1.27–1.95)	1.68 (1.33–2.12)	<0.001	
Male	1.41 (1.22–1.62)	<0.001	1 (ref.)	1.09 (0.90–1.33)	1.23 (0.98–1.55)	1.58 (1.30–1.93)	<0.001	
Current smoker								0.573
No	1.34 (1.21–1.48)	<0.001	1 (ref.)	1.15 (0.99–1.35)	1.47 (1.26–1.71)	1.67 (1.44–1.92)	<0.001	
Yes	1.52 (1.23–1.87)	<0.001	1 (ref.)	1.04 (0.75–1.45)	1.15 (0.80–1.65)	1.67 (1.17–2.40)	0.003	
BMI,kg/m^2								0.376
<30	1.31 (1.18–1.45)	<0.001	1 (ref.)	1.15 (0.98–1.36)	1.26 (1.05–1.53)	1.56 (1.33–1.82)	<0.001	
≥30	1.53 (1.30–1.81)	<0.001	1 (ref.)	1.00 (0.78–1.28)	1.62 (1.22–2.14)	1.81 (1.42–2.32)	<0.001	
eGFR, ml/min/1.73m^2								0.803
≥60	1.30 (1.17–1.46)	<0.001	1 (ref.)	1.10 (0.96–1.25)	1.29 (1.08–1.55)	1.46 (1.26–1.70)	<0.001	
<60	1.47 (1.23–1.76)	<0.001	1 (ref.)	1.75 (1.03–2.98)	2.37 (1.52–3.70)	3.01 (1.94–4.67)	<0.001	
Vit B12, pmol/L								<0.001
≥400	1.69 (1.35–2.12)	<0.001	1 (ref.)	1.13 (0.91–1.39)	1.40 (1.06–1.85)	1.79 (1.30–2.47)	0.001	
<400	1.25 (1.13–1.38)	<0.001	1 (ref.)	1.08 (0.89–1.31)	1.30 (1.07–1.58)	1.48 (1.21–1.81)	<0.001	
Hcy, μmol/L								0.646
<10	1.43 (1.20–1.71)	<0.001	1 (ref.)	1.16 (0.99–1.35)	1.32 (1.06–1.65)	1.48 (1.12–1.94)	0.003	
≥10	1.39 (1.25–1.56)	<0.001	1 (ref.)	1.01 (0.80–1.28)	1.32 (1.07–1.64)	1.52 (1.26–1.84)	<0.001	
Fasting status								0.877
Yes	1.39 (1.19–1.61)	<0.001	1 (ref.)	1.08 (0.83–1.41)	1.58 (1.18–2.12)	1.94 (1.47–2.55)	<0.001	
No	1.37 (1.20–1.56)	<0.001	1 (ref.)	1.12 (0.95–1.33)	1.27 (1.03–1.56)	1.46 (1.22–1.74)	<0.001	
Number of CV risk factors								0.379
≤1	1.35 (1.16–1.58)	<0.001	1 (ref.)	1.18 (0.95–1.47)	1.31 (1.02–1.68)	1.65 (1.34–2.03)	<0.001	
≥2	1.50 (1.20–1.86)	0.001	1 (ref.)	1.24 (0.89–1.73)	1.54 (0.96–2.44)	2.53 (1.57–4.07)	<0.001	

[a] HRs(95%CI) were assessed using weighted Cox proportional regression fully adjusted except for stratification factor. Values are hazard ratios (95% confidence interval). CV risk factor included current smoking, hypertension, dyslipidemia, and diabetes.

remained consistent in 2 subsets with an interval of 10-year (Fig. 2 and Table S6).

Incremental prognostic value of MMA in cardiovascular patients

Based on the above results, the prognostic value of MMA in participants with prior cardiovascular disease was investigated in NHANES 1999–2004 and 2011–2014 separately. Among 1387 patients with CVD from NHANES 1999–2004, MMA was added to the reference model and improved risk stratification of long-term mortality (Table 5). The likelihood ratio test suggested an improved model fit when MMA was added to the reference model ($p < 0.001$). The AIC and BIC were lower in the model included MMA, compared with the reference. Regarding discrimination, adding MMA to the reference model slightly increased the C-index for the prediction of all-cause mortality. In particular, the reference model plus MMA showed the highest improvement in reclassification for 10-year mortality as assessed by the continuous NRI 0.238 (0.141–0.330) and IDI 0.022(0.012–0.032), outmatched B12, homocysteine, and CRP. The incremental prognostic value of MMA for short-term mortality remained significant among 993 cardiovascular patients

Table 5
Prognostic value of baseline MMA for all-cause mortality among participants with CVD in NHANES 1999–2004 and 2011–2014.

Model	Reference	Reference + CRP	Reference + Cbl	Reference + Hcy	Reference + MMA
1999–2004 (n = 1387)					
Likelihood ratio test	–	<0.001	0.941	<0.001	<0.001
AIC	10823.7	10809.4	10825.7	10794.0	10766.8
BIC	10855.1	10846.0	10862.4	10830.6	10803.5
Harrell's C-index	0.724	0.730	0.724	0.731	0.735
Categorical NRI	–	0.005 (−0.013 to 0.019)[a]	−0.001 (−0.007 to 0.015)	0.014 (3.535–0.042)	0.044 (0.008–0.072)
Continuous NRI	–	0.031 (−0.011 to 0.149)	0.000 (−0.021 to 0.057)	0.150 (0.062–0.258)	***0.239 (0.141 to 0.330)***
event NRI	–	0.023 (−0.083 to 0.078)	0.000 (−0.029 to 0.049)	−0.011 (−0.047 to 0.039)	−0.005 (−0.057 to 0.063)
nonevent NRI	–	0.008 (−0.033 to 0.163)	0.000 (−0.010 to 0.031)	0.161 (0.065–0.270)	0.243 (0.179–0.316)
IDI	–	0.007 (0.002–0.014)	0.000 (0.000–0.002)	0.011 (0.004–0.017)	***0.022 (0.012 to 0.032)***
2011–2014 (n = 993)					
Likelihood ratio test	–	–	0.121	–	<0.001
AIC	1632.4	–	1632.0	–	1618.6
BIC	1661.8	–	1666.3	–	1652.9
Harrell's C-index	0.726	–	0.731	–	0.738
Categorical NRI	–	–	0.093 (−0.064 to 0.150)	–	0.142 (−0.042 to 0.293)
Continuous NRI	–	–	0.039 (−0.042 to 0.254)	–	***0.215 (0.028 to 0.429)***
event NRI	–	–	0.054 (−0.030 to 0.238)	–	0.155 (−0.010 to 0.313)
nonevent NRI	–	–	−0.016 (−0.030 to 0.032)	–	0.059 (−0.015 to 0.178)
IDI	–	–	0.005 (<0.001 to 0.035)	–	***0.015 (0.001 to 0.042)***

[a] Values are presented as point estimation and 95% confidence intervals. The reference model was derived from the Framingham risk score, consisting of age, sex, Charlson comorbidity index, systolic blood pressure, total cholesterol, and high-density lipoprotein-cholesterol. All statistics were estimated based on the unweighted Cox regression analysis. Each additional model is compared to the reference model. NRI and IDI are calculated for 10-year mortality in NHANES 1999-2004 and 2-year mortality in NHANES 2011-2014. AIC, Akaike information criterion; BIC, Bayesian information criterion; Cbl, vitamin B12; CRP, C-reactive protein; Hcy, homocysteine; IDI, integrated discrimination improvement; MMA, methylmalonic acid; NRI, net reclassification index.

in NHANES 2011–2014.

Discussion

In this large prospective study of 23,437 adults recruited from a nationally representative sample of the US, our findings demonstrated that higher levels of circulating MMA were significantly associated with increased all-cause and cardiovascular mortality, especially for those participants with normal cobalamin. Such a link remained existed independent of serum cobalamin, homocysteine, and CRP whether within 5-year or beyond 10-year follow-up. The relationship was repeatable in another subset of NHANES with an interval of 10 years. MMA showed an incremental prognostic value to predict short- and long-term mortality in cardiovascular patients based on the Framingham risk model, overmatched Hcy and CRP.

Several cross-sectional studies suggested that MMA was a potential risk factor of CVD, diabetic complications, and dementias. Compared with healthy controls, patients with myocardial infarction or heart failure had significantly higher MMA concentrations [17,25]. However, among 300 patients with AMI, higher MMA levels were insignificantly associated with adverse outcomes during a 4-year follow-up [26]. A limited sample size may hinder its power of test. Our results firstly demonstrated that MMA level at baseline was significantly associated with a higher risk of long-term all-cause and cardiovascular mortality in a large prospective cohort. Notably, MMA has a more excellent predictive value than Hcy or CRP in patients with cardiovascular disease. Further validation by hospital-based cohorts is required. Higher MMA was linked to the severity of diabetic peripheral neuropathy [27]. Serum methylmalonic acid also reflected the cognitive decline in older subjects [28]. In our current study, we did not find a robust relationship between MMA and mortality due to diabetes and Alzheimer's disease in the multivariable-adjusted model. Lower incidence might weaken the statistical effectiveness and bias the relation towards the null hypothesis.

However, all those studies still thought MMA as a marker of Cbl deficiency but not mitochondrial dysfunction. Mitochondria are considered as the critical regulatory center among multiple cellular processes [10]. Mitochondrial dysfunction characterized by impaired bioenergetics and redox imbalance is a notable hallmark of various chronic diseases including CVD and diabetes, which significantly influence health, disease development and progression, and life span [3,5]. Metabolism of MMA not only relies on healthy mitochondria and mitochondrial enzymes (MUT, CblA, and CblB), MMA accumulation also directly impacts on mitochondrial electron transport chain and redox status [11,13]. Currently, mitochondrial impairment induced by MMA and MMA-related metabolites is thought to be the major cause of mitochondrial ROS generation and redox imbalance in genetic methylmalonic acidemia [13]. A recent experimental study also demonstrated that mitochondrial dysfunction of kidney tubules was a critical pathogenic mechanism of MMA-related nephropathy in MUT knockout mice. Antioxidant therapy could partly attenuate the severity of nephropathy [16]. Thus, biological evidence supports MMA as a potential marker of mitochondrial impairment and redox disorder.

Moreover, accumulative observational studies had noted that cobalamin deficiency could only explain a small part of the increase of MMA in adults [29,30]. In our findings, the moderate correlations among blood Cbl, MMA, and Hcy also supported this point. Functional cobalamin deficiency was proposed to account for the status of elevated MMA and normal B12, which had been linked with oxidative stress and assumed to be caused by the oxidation of cobalamin [23]. However, healthy mitochondria and mitochondrial enzymes may be neglected, because the response to a large dose of Cbl treatment was largely inconsistent among subjects with functional B12 deficiency [11,23]. Besides, the insufficiency of functional Cbl should cause increases in both Hcy and MMA [11,12], whereas circulating MMA was moderately correlated to Hcy here ($r = 0.31$). Thus, a substantial part of the increased MMA level may be imputed to mitochondrial dysfunction [31,32]. Indeed, prior clinical studies supported the link between MMA and oxidative stress indicators in humans, such as oxidant risk factors, isoprostanes, oxidized cysteine, as well as decreased glutathione level and antioxidant capacity [33–35]. According to the analysis of fibroblasts derived from patients and controls, methylmalonic aciduria was accompanied by a decrease in mitochondrial respiration and abnormity in mitochondria morphology [36]. In our stratification analysis, a stronger association was identified between MMA (both continuous and categorical forms) and all-cause mortality in participants with B12 $\geq$ 400 pmol/L, compared with that in those with B12 $<$ 400 pmol/L. Thus, mitochondrial dysfunction and oxidative stress, as well as poor prognosis should be cautious in adults with elevated MMA, especially in

those with normal B12 simultaneously [17,23]. Those biological and clinical studies as well as our results support mitochondria-derived metabolite MMA as a promising surrogate biomarker for mitochondrial dysfunction and oxidative stress. Currently, MMA mainly served as a nutritional marker for monitoring B12 deficiency in adults, but not for predicting disease severity and poor prognosis. To the best of our knowledge, this is the first large prospective cohort study to demonstrate that circulating MMA is capable of predicting long-term all-cause and cardiovascular mortality independent of B12 and Hcy.

In contrast with Hcy, MMA may be an underestimated prognostic biomarker for CVD. Even though both MMA and Hcy were used to detect B12 deficiency, MMA had a more sensitive and specific performance than Hcy [11]. However, only Hcy has been endowed with clinical implications for CVD [24,37]. The moderate correlation between MMA and Hcy as well as the robust association of MMA with mortality independent of Hcy suggested that MMA as a prognostic marker could not be substituted by Hcy. Furthermore, our findings suggested MMA may improve risk stratification in cardiovascular patients outmatched Hcy. MMA-related metabolism occurred in mitochondria, while Hcy metabolized in cytoplasm [11]. As opposed to the complicated metabolism of Hcy, MMA was not subject to folate or vitamin B6 [26]. Our findings are of particular interest because the metabolism from MMA to succinate in mitochondria is potentially restorable, and therefore is more suitable to be a potentially modifiable biomarker than Hcy. Current evidence about the toxicity of MMA in mitochondrial impairment was almost based on congenital methylmalonic acidemia [13,38]. The underlying mechanisms in the setting of CVD and other chronic diseases need special clarification.

There is no universally accepted definition of the reference range of MMA. Several prior studies reported different ranges of plasma MMA values in normal participants, including 10–360 nmol/L, 90–250 nmol/L, <350 nmol/L, or <370 nmol/L [23,29,39,40]. Our results indicated that participants with MMA in the normal range (175–250 nmol/L) also had a 37% increased risk of mortality compared with those with MMA <120 nmol/L. The reference range of plasma MMA may need to be redefined. Fowler B et al. summarized the cause and diagnostic approach of methylmalonic acidurias [41]. In normal subjects, the ratio of urinary MMA on creatinine (uMMA/C) was commonly less than 10 mmol/mol [41,42]. The deficiency of each segment in the MMA-related metabolic process resulted in varying degrees of methylmalonic acidurias, ranging from mild elevation 10–20 mmol/mol to severe elevation over 20,000 mmol/mol. Generally, MMA level was higher in genetic disorders than Cbl deficiency [41]. Plasma MMA level was affected by renal impairment, while uMMA/C could limit the influence of the renal function [43]. Thus, uMMA/C may be a more suitable biomarker reflecting MMA-related metabolism, but robust evidence is required.

Quantification of downstream oxidative metabolites, such as thiols, malondialdehyde, 4-hydroxynonenal, and 8-hydroxy-2′-deoxyguanosine was vulnerable to the artifactual oxidation during the procedures of storage, isolation, and purification [44–46]. That limited their applicability to detect large-scale samples and merge multi-center data. Although some studies noted that MMA-related metabolites, such as 2-methylcitric acid and malonic acid, might act as more pivotal mediators than MMA itself in the pathogenesis of mitochondrial injury [38], the metabolic processes of MMA at least supported MMA as a surrogate marker of mitochondrial dysfunction [13]. Our results recommended further investigation of the biological mechanisms and predictive value in chronic disease, including CVD.

Limitations and strengths

This study may have some strength. The use of a nationally representative sample facilitates the generalization of our findings. The study population was divided into 2 parts, NHANES 1999–2004 and 2011–2014. We observed a striking and sustained association between MMA and mortality risk in two subsets that minimized the occasionality. Our study also has several limitations. First, MMA concentrations were analyzed using EDTA-plasma or serum specimens. Nevertheless, the potential difference may be ignorable as existing studies reported a common reference range and similar CV for MMA in serum and plasma [20,21]. Second, we could not distinguish inborn and postnatal increase of MMA. However, the incidence of hereditary methylmalonic acidemia was about 1/50,000–170,000 according to data from the US and Europe [47], which is unlikely to alter our conclusions. Third, we could not completely rule out the possibility of residual confounding unknown. Fourth, the causality could not be concluded due to the observational study design, whereas previous experimental studies demonstrated MMA-related metabolism critically mediates mitochondrial dysfunction and oxidative stress. Fifth, fasting status could slightly increase MMA concentrations. Even though the correlations between MMA and cardiometabolic markers as well as the associations between MMA and mortality in participants with fasting or not were consistent, further study should better use fasting blood for MMA detection.

Conclusions

Mitochondria-derived MMA is independently and robustly associated with increased all-cause and cardiovascular mortality in the general population, especially in participants with normal cobalamin. Such link extends beyond 10 years' follow-up. Moreover, circulating MMA improves risk stratification in patients with cardiovascular disease outmatched Hcy and CRP. Our results support MMA as a surrogate biomarker of mitochondrial dysfunction more than just monitoring cobalamin deficiency. The biological mechanisms under cardiovascular disease warrant further investigation.

Declaration of competing interest

The authors declare that there is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.redox.2020.101741.

Authors contributions

SW and BY conceived and designed the study. SF, BY, and YL developed the protocols. SW, YL, JL, and XZ organized all data. WT and HC analyzed and visualized the results. SW and YL contributed to the manuscript. Reviewed and edited the manuscript, BY and SF take the responsibility for the integrity and accuracy of this analysis.

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