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ORIGINAL RESEARCH

Homocysteine Metabolism, Subclinical Myocardial Injury, and Cardiovascular Mortality in the General Population

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ABSTRACT

BACKGROUND Homocysteine (Hcy) is a recognized cardiovascular disease (CVD) risk factor linked with atherosclerosis. However, the association between Hcy and myocardial injury is little known.

OBJECTIVES This study aimed to examine the associations between Hcy metabolism, subclinical myocardial injury, and cardiovascular mortality.

METHODS We included 10,871 participants without diagnosed CVD. Generalized linear regression was used to investigate the relationship between Hcy-related indicators (plasma total Hcy [tHcy], vitamin B_{12}, and folate) and myocardial injury biomarkers (high-sensitivity troponin T [hs-cTnT], high-sensitivity troponin I [hs-cTnI] measured using 3 assays [Abbott, Siemens, and Ortho], and N-terminal pro-B-type natriuretic peptide [NT-proBNP]).

RESULTS Among 10,871 participants, the weighted mean levels for tHcy, folate, and vitamin B_{12} were 8.58 μmol/L, 32.43 nmol/L, and 447.08 pmol/L, respectively. Plasma tHcy levels were positively associated with elevated hs-cTnT, hs-cTnI, and NT-proBNP, whereas folate and vitamin B_{12} were not inversely related to myocardial injury biomarkers. Multivariable-adjusted odds ratios for elevated hs-cTnT (19 ng/L) and NT-proBNP (125 pg/mL) per doubling of tHcy were 2.80 (95% CI: 1.17-6.73; $P < 0.001$) and 1.58 (95% CI: 1.20-2.08; $P < 0.001$), respectively. The associations of tHcy levels with elevated hs-cTnI (Abbott: 28 ng/L; Siemens: 46.5 ng/L; Ortho: 11 ng/L) were consistent. Indirect effects of tHcy on cardiovascular mortality risk via hs-cTnT and NT-proBNP explained up to 26.6% and 12.3% of the total effect, respectively.

CONCLUSIONS Plasma tHcy, not folate or vitamin B_{12}, is significantly associated with elevated hs-cTnT, hs-cTnI, and NT-proBNP in adults without CVD. Subclinical myocardial injury may substantially mediate Hcy-related cardiovascular mortality risk. (JACC Asia 2024;4:609-620) © 2024 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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ABBREVIATIONS AND ACRONYMS

CVD = cardiovascular disease

eGFR = estimated glomerular filtration rate

FP = fractional polynomial

Hcy = homocysteine

HHcy = hyperhomocysteinemia

HF = heart failure

hs-cTnI = high-sensitivity cardiac troponin I

hs-cTnT = high-sensitivity cardiac troponin T

LoD = limit of detection

NHANES = National Health and Nutrition Examination Survey

NT-proBNP = N-terminal pro-B-type natriuretic peptide

Q = quartile

SAH = *S*-adenosyl-homocysteine

tHcy = total homocysteine

Homocysteine (Hcy) is an established risk factor for cardiovascular disease (CVD).[1] Hyperhomocysteinemia (HHcy) is defined as an elevated plasma total Hcy (tHcy) concentration exceeding 15 μmol/L[2] and is associated with various vascular diseases, including hypertension, ischemic stroke, coronary heart disease, coronary artery calcification, and vascular dementia.[3] Epidemiologic studies have shown that HHcy prevalence in the United States is 6.9%,[4] whereas its distribution is higher in Asian countries, such as in China (37.2%)[5] and in Korean men (11.8%).[6] A recent meta-analysis of 11 prospective studies demonstrated that the risk of all-cause mortality increases by 33.6% for each 5 μmol/L of Hcy.[7] The B vitamins, including folate and vitamin B_{12}, are essential cofactors in Hcy metabolism. A deficiency in either of these vitamins may lead to an increase in Hcy levels, a contributing factor in the development of CVD.[8] However, previous studies have shown inconsistent results between Hcy levels, B vitamin supplementation, and CVD mortality.[9-14]

High-sensitivity cardiac troponin (hs-cTnT/I) has emerged as the preferred biomarker for detecting subclinical myocardial injury, enabling the estimation of troponin concentrations far below conventional clinical assay thresholds.[15] N-terminal pro-B-type natriuretic peptide (NT-proBNP), an inert fraction of the prohormone for B-type natriuretic peptide, is a robust biomarker for subclinical myocardial stress.[16] Numerous studies have demonstrated an association between elevated hs-cTnT/I and NT-proBNP levels and an increased likelihood of adverse cardiovascular events, including coronary artery disease and heart failure (HF), as well as all-cause and CVD mortality.[17-21] Although the relationship between Hcy and vascular injury has been established, studies elucidating the link between Hcy levels and myocardial injury remain scarce. To our knowledge, only one Chinese study reported the association of Hcy levels with hs-cTnT concentrations in an elderly, not middle-aged, adult population.[14] Nonetheless, the study had a relatively small sample size (n = 1,497), and the impact of other metabolic factors (ie, serum folate and vitamin B_{12} levels) was unknown.

To address these gaps, we examined the associations of Hcy-related biomarkers (including tHcy, folate, and vitamin B_{12}) with several biomarkers of subclinical myocardial damage (ie, hs-cTnT, hs-cTnI, and NT-proBNP) among 10,871 U.S. adults without CVD. Furthermore, we explored the mediation effect of myocardial injury biomarkers on the relationship between tHcy levels and cardiovascular mortality risk.

PATIENTS AND METHODS

STUDY POPULATION. This study used data from the National Health and Nutrition Examination Survey (NHANES), an ongoing series of cross-sectional sampling surveys conducted biennially by the National Center for Health Statistics of the Centers for Disease Control and Prevention.[22] All participants were individuals selected from the U.S. noninstitutionalized civilian population via a stratified, multistage, complex, and probability-based sampling design.[22]

In this study, we included 13,460 individuals aged 20 years or older with available data on plasma tHcy from NHANES 1999-2004. Additionally, we excluded participants with a CVD history, including self-reported cases of physician-diagnosed coronary heart disease, HF, and stroke (n = 1,418). Moreover, participants with missing hs-cTnT/I values (n = 1,035) were excluded. Consequently, data from 10,871 participants were included in the primary hs-cTnT analyses, along with the information from 10,863 and 10,848 participants for the hs-cTnI and NT-proBNP analyses, respectively (Supplemental Figure 1).

ETHICS APPROVAL AND CONSENT TO PARTICIPATE. All protocols were approved by the Institutional Ethics Board of the National Center for Health Statistics. All the participants provided written informed consent.

EXPOSURES: tHcy, FOLATE, AND VITAMIN B_{12}. The primary exposure plasma tHcy was measured using the Abbott Hcy assay, a fully automated fluorescence polarization immune assay technique developed by Abbott Diagnostics. In brief, dithiothreitol reduces the binding of Hcy with albumin and other molecules, releasing thiol groups. The addition of adenosine then facilitates the catalysis of Hcy to *S*-adenosyl-homocysteine (SAH) via SAH hydrolase. Subsequently, a specific monoclonal antibody and a fluoresceinated SAH analog tracer are used in the fluorescence polarization immune assay detection system. The plasma tHcy concentration is determined using the calibration curve stored on the Abbott AxSym immunoassay analyzer. This novel assay method is more effective than high-performance liquid chromatography for determining plasma tHcy levels.[23]

Next, we investigated the Hcy metabolism coenzymes serum folate and vitamin B_{12}.[24,25] The measurement of serum folate levels was conducted via

2 distinct methods during NHANES 1999 to 2004, that is, affinity/high-performance liquid chromatography with electrochemical (coulometric) detection in NHANES 1999 to 2000 and radioimmunoassay using the Bio-Rad Quantaphase II radioassay kit in NHANES 2001 to 2004. Finally, serum vitamin B_{12} levels were determined by the Bio-Rad Quantaphase II radioassay kit in NHANES 1999 to 2004. In these tests, the coefficient of variations was rigorously controlled within 10% and 5% for serum folate and B_{12} levels, respectively.

OUTCOMES: HS-cTnT, HS-cTnI, AND NT-proBNP. Hs-cTnT/I concentrations were measured using 4 assays on serum samples stored at the University of Maryland School of Medicine between 2018 and 2020.[26] Most stored serum samples (93%) had never undergone a freeze-thaw cycle.

Hs-cTnT levels in all participants were quantified using novel fifth-generation Elecsys assay reagents (Roche Cobas e601). The reported lower limit of detection (LoD) for Roche hs-cTnT is 3 ng/L.[26] Hs-cTnI levels were estimated using 3 commercial testing methods (Abbott AR-CHITECT i2000SR, Siemens Centaur XPT, and Ortho Vitros 3600), and all participants were repeatedly tested using 3 independent hs-cTnI assays. The LoDs of the Abbott, Siemens, and Ortho assays for hs-cTnI were 1.7, 1.6, and 0.39 ng/L, respectively.[26] Finally, NT-proBNP levels in the stored serum samples were measured using electrochemiluminescence immunoassay (Roche Diagnostics Corp) with a Cobas e601 analyzer (Elecsys, Roche Diagnostics). The LoD of this assay for NT-proBNP was 5 pg/mL.[17]

In our primary analyses, we defined elevated hs-cTnT/I levels based on concentrations above manufacturer-designated 99th percentiles for each assay, as specified by the International Federation of Clinical Chemistry and Laboratory Medicine.[26] Thus, elevated hs-cTnT level was defined as a concentration of ≥19 ng/L, and increased hs-cTnI levels in the Abbott, Siemens, and Ortho assays were defined as concentrations of ≥28 ng/L, ≥46.5 ng/L, and ≥11 ng/L, respectively.[17] Finally, we defined elevated NT-proBNP level as a concentration of ≥125 pg/mL.[18]

In the sensitivity analyses, we used alternative definitions to represent a myocardial injury with concentrations above these thresholds.[26] For men and women, the concentrations of hs-cTnT were 22 and 14 ng/L, and the thresholds of hs-cTnI for the Abbott, Siemens, and Ortho assays were 35 and 17 ng/L, 58 and 39.6 ng/L, 12 and 9 ng/L, respectively. Finally, we used the alternative definition of elevated NT-proBNP concentration (≥125 pg/mL for adults aged <75 years and ≥450 pg/mL for adults aged ≥75 years; ≥300 pg/mL; ≥450 pg/mL).[18]

CARDIOVASCULAR AND ALL-CAUSE MORTALITY. Participants in NHANES were linked to the National Death Index to ascertain their mortality status. Publicly available mortality data were compiled from the baseline assessment until either the date of death or December 31, 2019. The National Center for Health Statistics has published extensive guidelines and protocols for recording linkage and analyzing mortality data.[27] CVD-associated mortality was established according to the International Classification of Diseases-10th Revision, encompassing codes I00 to I09, I11, I13, and I20 to I51.

OTHER VARIABLES. See the Appendix for additional details.

STATISTICAL ANALYSES. Because of the complex sampling design of NHANES, all analyses in this study incorporated the primary sampling unit, pseudostrata, and sampling weights, unless otherwise specified. The characteristics of participants were presented as weighted mean with SD for continuous variables and percentages for categorical variables. Additionally, tHcy, folate, and vitamin B_{12} levels were log-transformed because of their skewed distribution. Plasma tHcy concentration was further stratified into quartile (Q) (Q1: <6.41; Q2: 6.42-7.89; Q3: 7.90-9.81; and Q4: ≥9.82 μmol/L). We performed a test for linear trend by entering the median value of each category of Hcy levels as continuous variables in the logistic regression models.

Spline curve fitting was conducted using fractional polynomial (FP) regression to flexibly evaluate potential nonlinear associations of tHcy, folate, and vitamin B_{12} with cardiac biomarkers. FP prediction plots were implemented with a default degree of 2 (FP1 and FP2) and a set of fractional powers (−2, −1, −0.5, 0, 0.5, 1, 2, 3), enabling a good description of a nonlinear association and avoiding model overfitting.[28] Automatic centering is reflected in the estimated regression coefficients and intercepts. Weighted multivariable logistic regression was used to determine the ORs and 95% CIs for assessing the associations between Hcy metabolism-related biomarkers (both continuous and by quartile) and elevation in hs-cTnT, hs-cTnI, and NT-proBNP levels. Because of skew and discrete distribution, tHcy, vitamin B_{12}, and folate were $\log_2$ transformed. The OR of elevated cardiac biomarkers for each unit increase in $\log^2$-transformed tHcy could be interpreted as the increased odds for each doubling of tHcy.[29] Subsequently, several adjusted

models were developed: 1) model 1 included adjustments for age, sex, and race/ethnicity; 2) model 2 integrated the covariates from model 1 plus smoking status, alcohol use, body mass index, diabetes, hypertension, high-density lipoprotein cholesterol, aspartate aminotransferase, alanine aminotransferase, C-reactive protein (CRP), and estimated glomerular filtration rate (eGFR) data; and 3) model 3 for tHcy was further adjusted for folate and vitamin B_{12} levels, and vice versa. After adjustment for the potential confounding factors included in model 3, Cox proportional hazards regression models were used to estimate the HRs for mortality risk according to the plasma tHcy and cardiac biomarkers. Moreover, concentrations lower than the LoDs of each assay were included in all regression models to standardize the different assays. This step was undertaken because although hs-cTn concentrations were found to be lower than the LoDs, these lower concentrations have been reported to contain useful prognostic information.[30]

Stratified analyses were performed in the strata of age (<65 or ≥65 years), sex (female or male), race (White or non-White), smoking (no or yes), body mass index (<30 or ≥30 kg/m^2), hypertension (no or yes), diabetes (no or yes), eGFR (<60 or ≥60 mL/min/1.73 m^2), vitamin B_{12} (<295 or ≥295 pmol/L), and folate (<21.3 or ≥21.3 nmol/L). The *P* values for the interaction of tHcy levels and stratified variables were calculated.

Additionally, we conducted a series of sensitivity analyses. First, we categorized plasma tHcy levels into 2 groups based on commonly used cutoffs (<15 μmol/L and ≥15 μmol/L).[2] Second, we broadly defined elevated hs-cTnT, hs-cTnI, and NT-proBNP levels according to sex-specific reference values to further establish the relationship between tHcy levels and cardiac biomarkers. Third, we used the 75th, 97.5th, and 99th percentile values of hs-cTnT, hs-cTnI, and NT-proBNP as the cutoffs to explore the congruence with the primary analysis findings. Then, weighted Pearson correlation coefficients were calculated between $\log_2$-transformed Hcy metabolism and cardiac biomarkers.

In mediation analyses, we aimed to assess the degree to which subclinical myocardial injury biomarkers (mediator: ie, hs-cTnT, hs-cTnI, and NT-proBNP) mediate the association between plasma tHcy (exposure) and mortality (outcome) after adjustment for the potential confounders included in model 3. We set 3 pathways for this purpose: briefly, 1) exposure to mediator; 2) mediator to outcome (direct effect); and 3) exposure to outcome (total effect). The difference between the total effect and the direct effect is called the mediated (indirect) effect, which is the partial effect of mediating variables replacing independent variables to explain the dependent variable. The mediated proportion was computed using the following formula: the natural indirect effect divided by the natural total effect. Significance testing of mediation analysis was performed using a bootstrap method.

All analyses were performed using Stata version 15.1 (Stata Corp,). Two-sided *P* values of <0.05 were considered statistically significant.

RESULTS

CHARACTERISTICS OF THE STUDY POPULATION. Our analysis included 10,871 participants without a history of CVD (mean age: 44.6 years; SD: 12.58; 47.7% male; 71.8% White), as shown in **Table 1**. The mean tHcy, folate, and vitamin B_{12} levels of the participants were 8.58 μmol/L, 32.43 nmol/L, and 447.08 pmol/L, respectively. Compared with the participants with lower tHcy levels, those having higher tHcy levels were more likely to be older, male, non-Hispanic white, and nonsmokers and showed a higher prevalence of hypertension and diabetes as well as lower serum vitamin B_{12} and eGFR levels.

HCY METABOLISM AND SUBCLINICAL MYOCARDIAL INJURY BIOMARKERS. According to the FP regression analysis, we found that plasma tHcy levels were positively associated with hs-cTnT, hs-cTnI, and NT-proBNP levels (**Figure 1**). The observed shapes of the relationships of serum folate levels with hs-cTnT, hs-cTnI, and NT-proBNP were consistently similar. Higher serum folate was associated with greater myocardial injury, with a notable inflection point at a serum folate concentration above 64 nmol/L. However, the vitamin B_{12} level was found to correlate with NT-proBNP concentration only at a B_{12} level exceeding 1,000 pmol/L.

In the multivariable logistic regression analyses, higher plasma tHcy levels were associated with higher odds of elevated hs-cTnT and NT-proBNP concentrations, with this result persisting even after adjustment for the potential confounding factors (**Table 2**). Compared with the reference group (Q1), the adjusted ORs from Q1 to Q4 of plasma tHcy concentration for elevated hs-cTnT levels were 1.00 (reference), 0.88 (95% CI: 0.33-2.35), 1.34 (95% CI: 0.53-3.42), and 2.80 (95% CI: 1.17-6.73), with a *P* for trend of <0.001, and for elevated NT-proBNP the

TABLE 1 Clinical Characteristics of 10,871 Participants Without CVD Across tHcy Quartiles, NHANES 1999-2004

		Plasma tHcy Levels, μmol/L				
	Total (N = 10,871)	Quartile 1 (<6.41) (n = 2,728)	Quartile 2 (6.42-7.89) (n = 2,708)	Quartile 3 (7.90-9.81) (n = 2,719)	Quartile 4 (≥9.82) (n = 2,716)	*P* Value for Trend
Age, y	44.6 ± 12.58	48.6 ± 9.40	56.2 ± 10.10	59.1 ± 9.72	64.7 ± 10.35	<0.001
Male	5,070 (47.7)	518 (22.2)	1,238 (44.8)	1,607 (59.5)	1,707 (63.1)	<0.001
Race/ethnicity						<0.001
Non-Hispanic White	5,447 (71.8)	1,160 (64.3)	1,317 (71.6)	1,471 (75.4)	1,499 (75.4)	
Non-Hispanic Black	1,958 (9.8)	423 (9.9)	472 (9.5)	521 (9.8)	542 (10.3)	
Hispanic	2,545 (7.6)	858 (12.1)	693 (8.4)	522 (5.7)	472 (4.4)	
Other ethnicity	921 (10.7)	287 (13.7)	226 (10.5)	205 (9.9)	203 (9.9)	
Smoking status						<0.001
Never smoker	5,710 (51.1)	1,727 (60.7)	1,459 (52.2)	1,329 (48.6)	1,195 (43.1)	
Former smoker	2,710 (24.0)	526 (19.5)	638 (24.0)	711 (24.6)	835 (27.8)	
Current smoker	2,434 (24.9)	470 (19.8)	608 (23.7)	676 (26.8)	680 (29.2)	
Alcohol consumption, g/d	5.0 ± 9.43	1.9 ± 4.70	2.1 ± 4.71	3.2 ± 8.13	4.1 ± 8.43	<0.001
Physical activity						<0.001
Inactive	4,646 (35.0)	1,120 (32.7)	1,057 (31.1)	1,134 (35.4)	1,335 (41.5)	
Moderate	3,025 (29.2)	776 (28.1)	717 (28.3)	754 (30.2)	778 (30.2)	
Vigorous	3,199 (35.8)	832 (39.3)	934 (40.6)	830 (34.4)	603 (28.4)	
BMI, kg/m^2	28.0 ± 4.87	33.0 ± 5.66	32.3 ± 5.55	31.2 ± 5.11	31.0 ± 6.48	0.143
Hypertension	3,989 (31.7)	543 (18.9)	862 (27.3)	1045 (33.5)	1,539 (47.9)	<0.001
Diabetes	988 (6.4)	171 (5.1)	216 (5.4)	223 (5.4)	378 (9.8)	<0.001
TC, mmol/L	5.2 ± 0.83	5.2 ± 0.76	5.4 ± 0.85	5.4 ± 0.98	5.2 ± 1.07	<0.001
HDL-C, mmol/L	1.4 ± 0.32	1.2 ± 0.24	1.2 ± 0.24	1.2 ± 0.27	1.3 ± 0.35	<0.001
LDL-C, mmol/L	3.1 ± 0.66	3.0 ± 0.60	3.1 ± 0.56	3.1 ± 0.68	2.8 ± 0.66	<0.001
ALT, U/L	26.3 ± 24.51	31.1 ± 22.53	28.6 ± 13.77	31.8 ± 23.89	29.3 ± 21.13	0.009
AST, U/L	28.9 ± 14.54	26.4 ± 23.65	25.3 ± 9.35	27.1 ± 13.07	30.1 ± 26.23	<0.001
Plasma glucose, mmol/L	5.2 ± 1.19	9.2 ± 3.13	8.2 ± 2.73	7.9 ± 3.06	8.3 ± 3.62	<0.001
C-reactive protein, mg/dL	0.4 ± 0.61	0.7 ± 0.59	0.7 ± 1.16	0.5 ± 0.48	0.6 ± 0.95	0.343
eGFR, mL/min per 1.73 m^2	100.0 ± 17.16	106.6 ± 12.58	93.0 ± 13.38	86.2 ± 14.23	67.5 ± 19.93	<0.001
Serum B_{12}, pmol/L	396.6 ± 1,034.46	452.7 ± 95.91	398.6 ± 142.23	368.9 ± 129.15	343.2 ± 199.26	<0.001
Serum folate, nmol/L	32.3 ± 19.41	34.8 ± 13.77	42.1 ± 74.56	34.0 ± 15.15	34.8 ± 20.17	<0.001

Values are n (%) or weighted mean ± SD.

ALT = alanine aminotransferase; AST = aspartate aminotransferase; BMI = body mass index; CVD = cardiovascular disease; eGFR = estimated glomerular filtration rate; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein-cholesterol; NHANES = National Health and Nutrition Examination Survey; TC = total cholesterol; tHcy = total homocysteine.

levels were 1.00 (reference), 1.03 (95% CI: 0.76-1.40), 1.09 (95% CI: 0.85-1.40), and 1.58 (95% CI: 1.20-2.08), with a *P* for trend of <0.001. Similar correlations were observed for the Abbott and Ortho assay results of hs-cTnI. However, this association was markedly attenuated for the elevated hs-cTnI levels (*P* for trend = 0.068) of the Siemens assay after adjustment for age, sex, and race/ethnicity (Supplemental Table 1).

Serum folate level was significantly associated with higher odds of cardiac damage, with the OR per doubling of folate level being 1.54 (95% CI: 1.33-1.78; $P < 0.001$), 1.73 (95% CI: 1.60-1.86; $P < 0.001$), 1.54 (95% CI: 1.17-2.02; $P = 0.003$), and 1.67 (95% CI: 1.18-2.37; $P = 0.005$) for elevated concentrations of hs-cTnT, NT-proBNP, hs-cTnI (Siemens), and hs-cTnI (Ortho), respectively (Supplemental Table 2). However, these associations were not statistically significant after adjustment for age, sex, and race/ethnicity. Additionally, serum vitamin B_{12} levels were not significantly associated with elevated hs-cTnT and hs-cTnI concentrations (Supplemental Table 3).

Stratification analyses based on the potential confounders of the relationship between plasma tHcy and subclinical myocardial injury biomarkers are presented in Supplemental Figures 2 to 4. The analyses showed a significant interaction between plasma tHcy and eGFR levels in elevated hs-cTnT and NT-proBNP levels. Compared to participants with eGFR of ≥60 mL/min/1.73 m^2, those with eGFR of <60 mL/min/1.73 m^2 have higher ORs per doubling of tHcy concentration for elevated hs-cTnT (5.07 vs 2.12; *P* for interaction = 0.013) and NT-proBNP (2.13 vs 1.45; *P* for interaction = 0.018) levels. Moreover,

FIGURE 1 **Relationship Between Hcy Metabolism-Related Indicators and Cardiac Biomarkers**

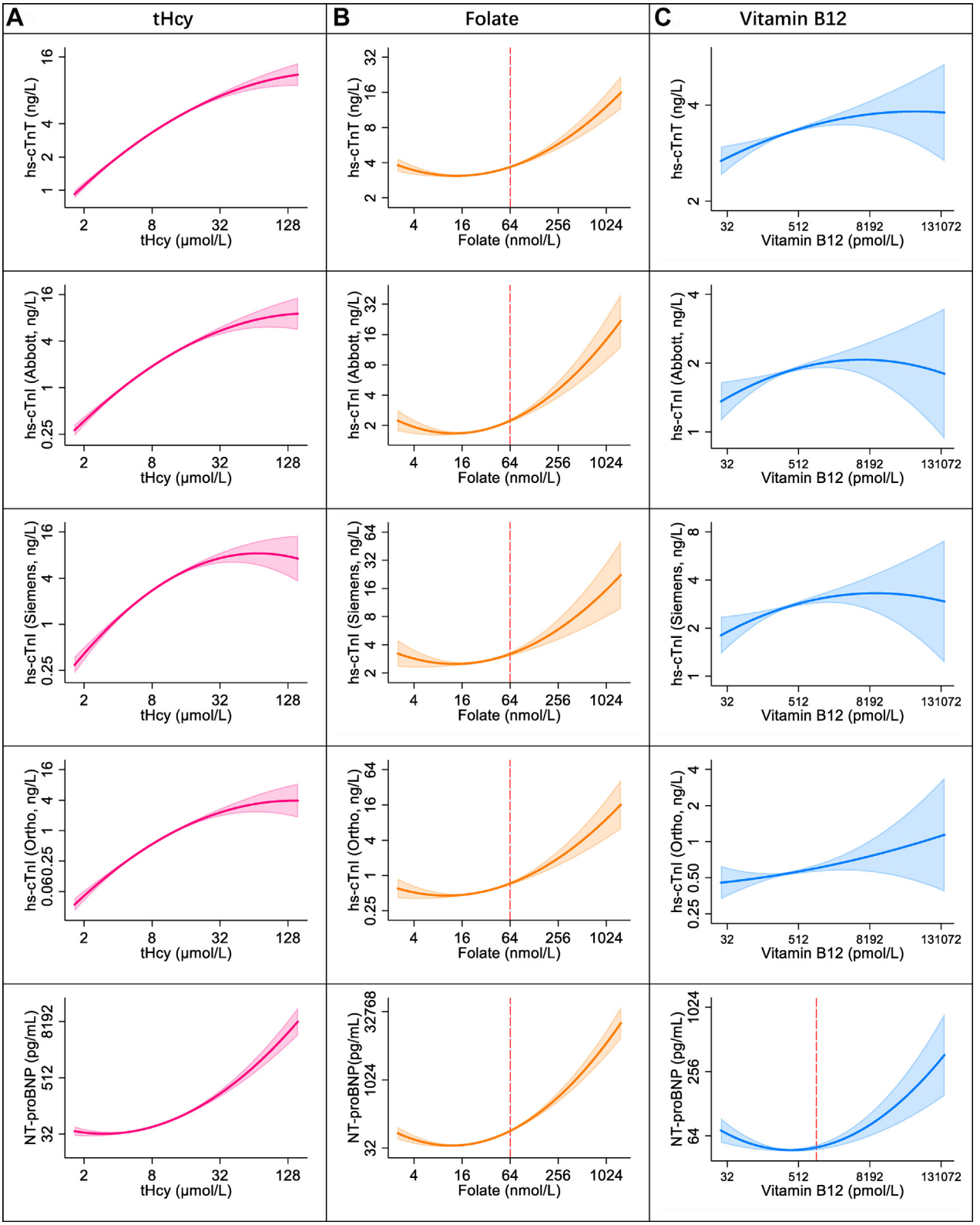

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TABLE 2 The ORs (95% CIs) Associated With tHcy Quartiles for Elevated hs-cTnT and NT-proBNP Levels

	Doubling in tHcy	*P* Value	Q1	Q2	Q3	Q4	*P* Value for Trend
Elevated hs-cTnT							
Events	545 (5.01)		13 (0.48)	42 (1.55)	102 (3.75)	388 (14.29)	
Crude model	5.54 (4.32-7.09)	<0.001	1 (ref)	1.77 (0.72-4.37)	4.46 (1.87-10.63)	19.52 (8.76-43.50)	<0.001
Model 1	3.30 (2.48-4.40)	<0.001	1 (ref)	0.92 (0.36-2.37)	1.42 (0.59-3.42)	3.63 (1.56-8.46)	<0.001
Model 2	2.57 (1.96-3.36)	<0.001	1 (ref)	0.87 (0.33-2.31)	1.30 (0.52-3.30)	2.66 (1.12-6.33)	<0.001
Model 3	2.67 (2.03-3.51)	<0.001	1 (ref)	0.88 (0.33-2.35)	1.34 (0.53-3.42)	2.80 (1.17-6.73)	<0.001
Elevated NT-proBNP							
Events	2,023 (18.65)		229 (8.41)	351 (13.00)	468 (17.26)	975 (35.95)	
Crude model	2.78 (2.40-3.22)	<0.001	1 (ref)	1.31 (1.01-1.70)	1.64 (1.35-1.99)	3.76 (3.02-4.70)	<0.001
Model 1	1.78 (1.50-2.12)	<0.001	1 (ref)	0.99 (0.74-1.33)	1.06 (0.84-1.35)	1.87 (1.45-2.42)	<0.001
Model 2	1.47 (1.24-1.75)	<0.001	1 (ref)	1.03 (0.76-1.40)	1.08 (0.84-1.40)	1.56 (1.19-2.06)	<0.001
Model 3	1.49 (1.25-1.77)	<0.001	1 (ref)	1.03 (0.76-1.40)	1.09 (0.85-1.40)	1.58 (1.20-2.08)	<0.001

Values are n (%) or OR (95% CI) unless indicated otherwise. The elevation in hs-cTnT and NT-proBNP were defined as a concentration of ≥19 ng/L and ≥125 pg/mL, respectively. ORs (95% CIs) were calculated by weighted logistic regression model. Model 1: age, sex, race/ethnicity. Model 2: variables in model 1 plus smoking, alcohol use, physical activity, body mass index, diabetes, hypertension, total cholesterol, high-density lipoprotein cholesterol, aspartate aminotransferase, alanine aminotransferase, C-reactive protein, and estimated glomerular filtration rate. Model 3: variables in model 2 plus folate and vitamin B_{12}.

hs-cTnT = high-sensitivity cardiac troponin T; NT-proBNP = N-terminal pro-B-type natriuretic peptide; Q = quartile; ref = reference; tHcy = total homocysteine.

compared to participants without diabetes, those with diabetes had higher odds of elevated hs-cTnT (9.16 vs 2.31; *P* for interaction = 0.016) and hs-cTnI (Ortho assay: 8.43 vs 1.88; *P* for interaction = 0.011) levels. However, no significant interactions were observed for the associations of the serum folate, vitamin B_{12}, and tHcy levels with subclinical myocardial injury.

Additionally, we observed similar results in sensitivity analyses based on sex-specific reference ranges (Supplemental Table 4). Also, an analysis examining the tHcy level as a categorical variable observed no notable changes in the association between tHcy and myocardial injury biomarkers (Supplemental Table 5). A cutpoint analysis further showed consistent and robust associations of plasma tHcy levels with elevated hs-cTnT concentrations (Supplemental Table 6). In addition, compared with vitamin B_{12} and folate, serum tHcy showed higher correlation coefficients with cardiac biomarkers (Supplemental Table 7).

MEDIATION OF HCY AND MORTALITY ASSOCIATIONS THROUGH HS-cTnT. Initially, we demonstrated that plasma tHcy and subclinical myocardial biomarkers were associated with mortality (Supplemental Table 8). Comparison of the HRs for CVD mortality between these biomarkers showed that Hcy (HR: 1.53; 95% CI: 1.29-1.81) and hs-cTnT (HR: 1.87; 95% CI: 1.63-2.14) had higher HRs than hs-cTnI and NT-proBNP. Next, we conducted a mediation analysis to explore the indirect effects of independent cardiac biomarkers on the associations between plasma tHcy levels and mortality outcomes (**Figure 2**). In this analysis, the total indirect role for 5 myocardial injury biomarkers was calculated with β1 to β10. Our results suggest that hs-cTnT mediated 26.6% and 23.0% of the effects on CVD and all-cause mortality, respectively, and that NT-proBNP mediated 7.6% and 12.3% of the effects on CVD and all-cause mortality, respectively.

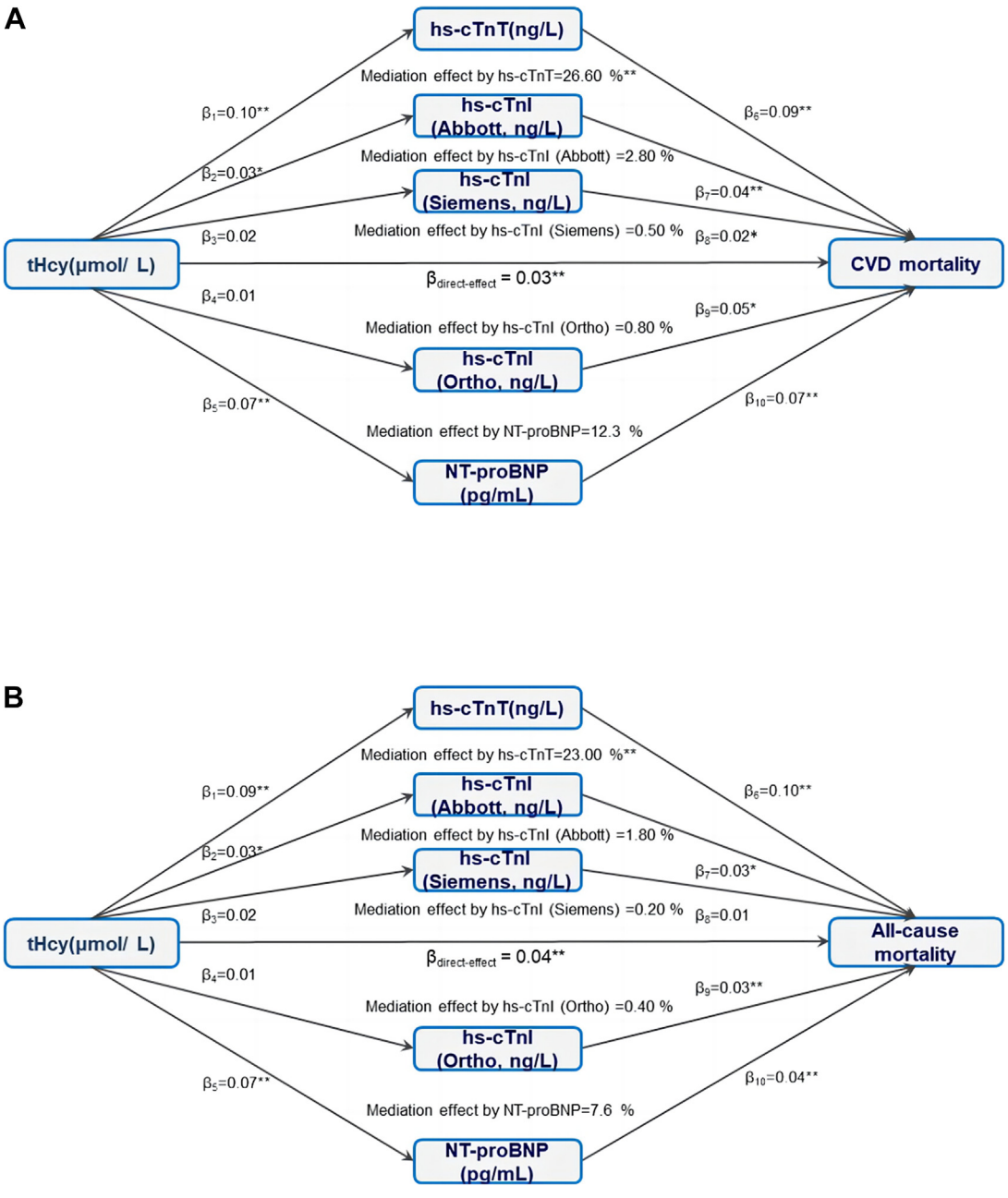
DISCUSSION

Our results show that plasma tHcy levels were positively associated with subclinical myocardial injury, as reflected by hs-cTnT, hs-cTnI, and NT-proBNP levels. However, serum folate and vitamin B_{12} concentrations were not associated with a reduced likelihood of subclinical myocardial injury. Additionally,

FIGURE 1 Continued

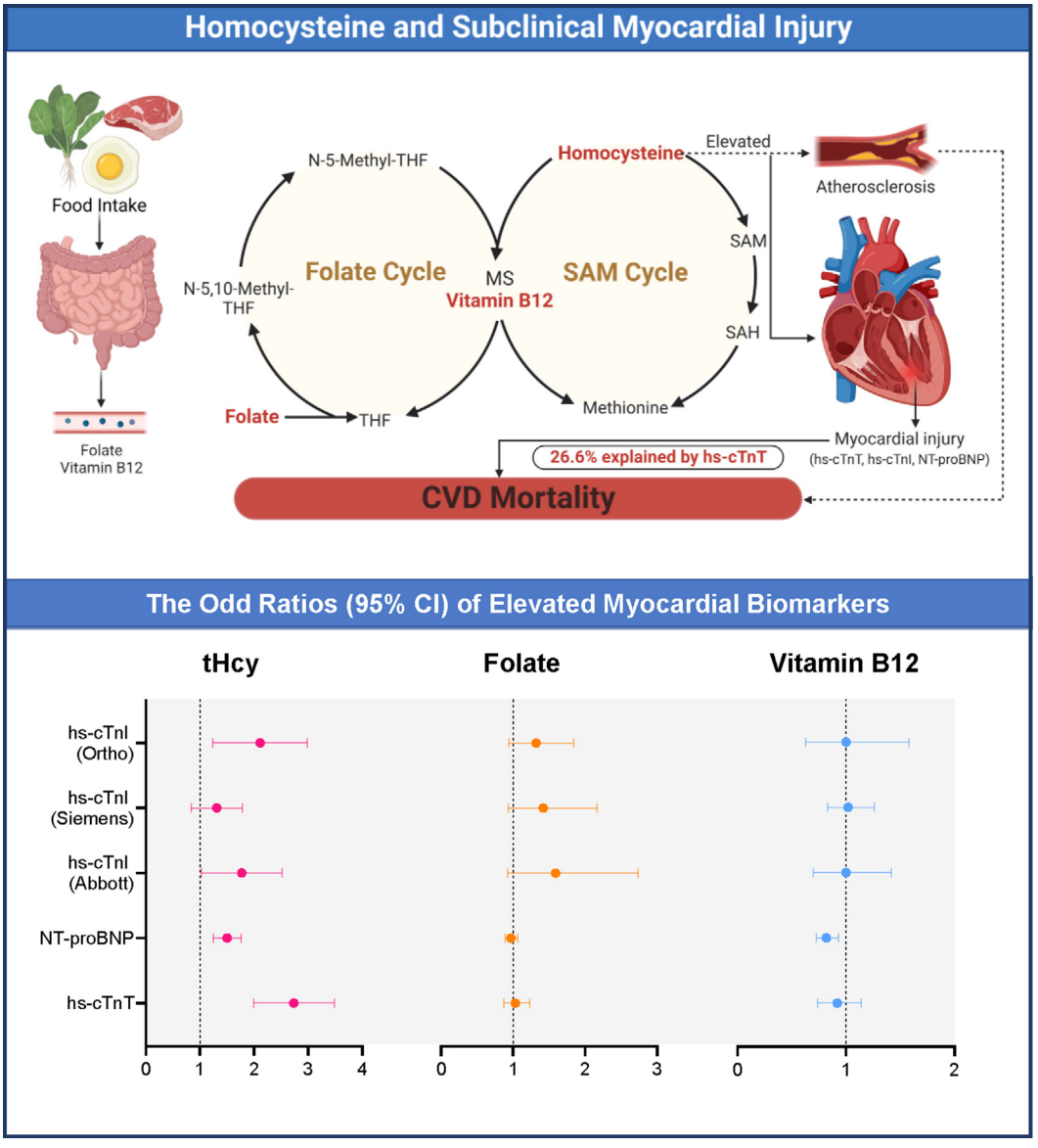
The graphs show the nonlinearity of the association of (A) tHcy, (B) folate, and (C) vitamin B12 with subclinical myocardial injury biomarkers (hs-cTnT, 3 assays for hs-cTnI, and NT-proBNP). The red-, yellow-, blue-shaded regions show the 95% CIs around the regression line. The red dashed lines represent folate of 64 nmol/L and vitamin B_{12} of 1,000 pmol/L. hs-cTnI = high-sensitivity cardiac troponin I; hs-cTnT = high-sensitivity cardiac troponin T; NT-proBNP = N-terminal pro-B-type natriuretic peptide; tHcy = total homocysteine.

FIGURE 2 Mediation Analysis for the Association Between tHcy and Mortality Risk



(A) The mediation analysis for the association between plasma tHcy and CVD mortality. (B) The mediation analysis for the association between plasma tHcy and all-cause mortality. The association with mortality risk was estimated with weighted Cox regression analysis, adjusted for age, sex, race/ethnicity, smoking, alcohol use, physical activity, body mass index, diabetes, hypertension, total cholesterol, high-density lipoprotein cholesterol, aspartate aminotransferase, alanine aminotransferase, C-reactive protein, estimated glomerular filtration rate, folate, and vitamin B_{12}. Arrows indicate the effect pathway. *$P < 0.05$; **$P < 0.01$. CVD = cardiovascular disease; other abbreviations as in Figure 1.

CENTRAL ILLUSTRATION **Associations Between Homocysteine Metabolism, Subclinical Myocardial Injury, and Cardiovascular Mortality**



Tan X, et al. JACC Asia. 2024;4(8):609–620.

Circulating Hcy, not serum folate or vitamin B_{12}, was associated with subclinical myocardial injury. The elevation in serum hs-cTnT substantially mediated the association of Hcy with cardiovascular mortality risk. Multivariable-adjusted ORs per doubling of circulating Hcy, folate, and vitamin B_{12} were estimated by logistic regression analysis after considering sampling weights. CVD = cardiovascular disease; Hcy = homocysteine; hs-cTnI = high-sensitivity cardiac troponin I; hs-cTnT = high-sensitivity cardiac troponin T; MS = methionine synthase; NT-proBNP = N-terminal pro-B-type natriuretic peptide; SAH = *S*-adenosylhomocysteine; SAM = *S*-adenosylmethionine; THF = tetrahydrofolate.

hs-cTnT was found to have a mediating effect of 26.6% on the association between plasma tHcy and CVD mortality (Central Illustration). Those findings suggest that myocardial injury related to Hcy accumulation might not be readily reversible with folate and vitamin B_{12} supplementation. To our knowledge, this is the first study to examine the associations between Hcy metabolism-related

biomarkers with subclinical myocardial injury in adults without a history of CVD.

HHcy is an independent risk factor for various vascular diseases, such as atherosclerosis, hypertension, vascular calcification, and aneurysm (including abdominal aortic aneurysm, intracranial aneurysm, ascending aortic aneurysm, and thoracic aortic aneurysm),[3] which has been demonstrated by numerous animal and human studies.[1,3,31] Current knowledge on Hcy is primarily focused on its relationship with vascular injury, with few studies examining the association of Hcy with myocardial injury. Our study expands the conventional understanding of Hcy in clinical practice and represents the first attempt to assess the association between Hcy metabolism and subclinical myocardial injury biomarkers within a nationally representative sample. To the best of our knowledge, only one prior study examined the relationship between serum Hcy and elevated hs-cTnT concentrations among 1,497 participants without a history of CVD. Higher serum Hcy levels were linked with a higher likelihood of detectable hs-cTnT levels in elderly individuals, not in adults <65 years old.[14] The sample size may limit the statistical power of their analysis. Moreover, integrating Hcy metabolism-related biomarkers beyond just biochemically detecting circulating Hcy levels may provide additional information for clinical practice. It is important to note that the cardiovascular benefits of folic acid or vitamin B_{12} supplementation have been debated.[10-13,29] In the present study, we used a larger sample size of 10,871 U.S. adults without a history of CVD. Our study shows that high plasma tHcy levels were significantly associated with a greater likelihood of myocardial injury, whereas serum folate and vitamin B_{12} concentrations were not associated with a reduced likelihood of subclinical myocardial injury.

The associations between serum folate, vitamin B_{12}, and CVD mortality in previous studies were largely controversial. For example, some studies found that higher serum folate was associated with a decreased risk of adverse cardiovascular events,[11] but the inverse association was also reported.[12] In our analysis, the positive association between serum folate and elevated cardiac biomarkers was significant in the unadjusted logistic regression model but became insignificant after multivariable adjustment. Biologically, excess folate may dysregulate Hcy metabolism by reducing methylenetetrahydrofolate reductase activity and disturbing the balance between thymidylate synthase and methionine synthase.[32] Previous cohort studies found that higher concentrations of vitamin B_{12} were significantly associated with increased risk of mortality and other adverse outcomes.[13] We previously conducted studies in patients with diabetes or coronary heart disease to investigate cobalamin supplementation or serum concentration and mortality risk, and the results were negative.[10,29] Moreover, we reported robust associations between methylmalonic acid, another biomarker of vitamin B_{12} deficiency, and cardiovascular mortality, which was even more strengthened in adults with higher serum vitamin B_{12} levels.[10] A compensatory response to decreased cobalamin sensitivity may account for this phenomenon as observed in some cases of methylmalonic acidemia that is not consistently corrected with high-dose cyanocobalamin therapy.[29] Despite a significant association of Hcy accumulation with elevated cardiac biomarkers observed in the current study, serum folate and vitamin B_{12} were not associated with a reduced likelihood of myocardial injury. Those results were consistent with a previous report that Hcy accumulation may be not consistently corrected by large-dose supplementation of B vitamins.[33] One of the explanations may be the toxicity of cyanocobalamin among participants with renal failure. Using B vitamins to reduce cardiovascular risk should use methylcobalamin and 5-methyltetrahydrofolate instead of cyanocobalamin.[34] Further study is warranted to elucidate the mechanisms.

Elevated hs-cTn is an established biomarker of myocardial injury, predicting the risk of HF and CVD mortality among asymptomatic individuals and individuals with coronary heart disease.[17,35] We also found that tHcy levels were positively associated with NT-proBNP concentrations. Our results are in line with the reports in prior work.[36] However, renal failure, systemic inflammatory response, and endocrine disease may lead to elevation in cardiac biomarkers. In the current study, we demonstrated a robust and independent association between tHcy and hs-cTnT/I and NT-proBNP levels among participants without a history of atherosclerotic CVD, after adjustment for eGFR, C-reactive protein, and liver enzymes. According to a previous biological study, the link between tHcy and subclinical myocardial injury may involve an increase in cellular oxidative injury caused by the production of reactive oxygen species.[37] In addition, we found that, compared to hs-cTnI levels, hs-cTnT concentrations may have a stronger association with tHcy levels. Three hs-cTnI assays are heterogeneously affected by macrotroponin, heterophile

antibodies, and spuriously elevated results. According to the manufacturer-reported threshold of hs-cTn assays, a smaller proportion of adults with elevated hs-cTnI compared to those with increased hs-cTnT may limit the performance of hs-cTnI. Another possible explanation for this finding could be the degradation of circulating TnI in chronic myocardial injury. Nils et al[38] compared the cardiac troponin assays and found that the hs-cTnI/hs-cTnT ratio was higher in 410 participants with acute myocardial injury than in 467 individuals with chronic myocardial injury. Our results from the general population support that hs-cTnT might provide greater information for chronic subclinical myocardial injury.[17]

STUDY LIMITATIONS. First, the cross-sectional design of our study prevents the establishment of a causal relationship between tHcy and subclinical myocardial injury. We relied on cardiac biomarkers to detect subclinical myocardial injury, with no additional methods (eg, echocardiographic strain and cardiac magnetic resonance for determining subclinical myocardial damage. Compared to strain imaging or cardiac magnetic resonance, cardiac biomarkers (hs-cTnI/T or NT-proBNP) are more widely used to reflect myocardial injury and predict adverse cardiovascular events in populations without clinical CVD.[39] Second, the cofactors of Hcy metabolism, including vitamin B_{12} and folate, have been suggested to modulate the level of Hcy.[8] Additionally, measurements at multiple timepoints may provide more information. Third, our study assessed hs-cTn-associated myocardial injury by applying manufacturer-defined thresholds to the full age range of adults; however, the thresholds provided by these manufacturers might change over time. Finally, we could not completely exclude the residual or unknown confounding factors despite extensive adjustments for potential confounding factors in our final model. Nevertheless, our study has some strengths. The present study was based on a nationally representative sample of U.S. adults who had no history of CVD. Troponin levels were assessed using multiple high-sensitivity assays, enabling comprehensive comparisons and the generalization of the findings.

CONCLUSIONS

In this study, we comprehensively investigated the association of Hcy metabolism-related biomarkers, subclinical myocardial injury, and cardiovascular mortality in adults without a history of CVD. Our results revealed that plasma tHcy (not serum folate or vitamin B_{12}) was associated with a greater likelihood of subclinical myocardial injury, as reflected by hs-cTnT, hs-cTnI, and NT-proBNP levels. Subclinical myocardial injury may substantially mediate the association between plasma tHcy levels and CVD mortality risk. Further studies should explore other strategies to improve Hcy metabolism more than simply supplementing folate or vitamin B_{12} to promote cardiovascular health.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Circulating Hcy, not serum folate or vitamin B_{12}, was associated with subclinical myocardial injury. Moreover, elevated hs-cTnT substantially mediated the association of tHcy with cardiovascular mortality risk.

TRANSLATIONAL OUTLOOK: Considering the high prevalence of elevated Hcy in Asian populations, future validation in large-scale representative Asian populations is highly desirable. Myocardial injury related to Hcy may not be attributed to folate or vitamin B_{12} deficiency, and thus, further studies should explore other strategies to improve Hcy metabolism more than simply supplementing folate or vitamin B_{12} to promote cardiovascular health.

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KEY WORDS cardiac biomarker, cobalamin, folate, Hcy, homocysteine, subclinical myocardial injury, vitamin B_{12}

APPENDIX For supplemental tables and figures, and an expanded Methods section, please see the online version of this paper.