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ORIGINAL RESEARCH

Choline Metabolites, Genetic Susceptibility, and Incident Heart Failure

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ABSTRACT

BACKGROUND Little is known about the associations between choline metabolites (total choline, phosphatidylcholine, and glycine) and the incidence of heart failure (HF).

OBJECTIVES The purpose of this study was to assess the associations of choline metabolites with incident HF and examine the effect modification by genetic susceptibility.

METHODS This prospective cohort study followed 245,072 participants from the UK Biobank from baseline (2006-2010) until March 30, 2023. Participants were free of cardiovascular diseases at baseline. Circulating choline metabolites were quantitated using nuclear magnetic resonance spectrometer. Cox proportional hazards models were fitted to assess the association of choline metabolites and genetics with incident HF. Two-sample Mendelian randomization analyses were implemented to confirm the findings in observational analysis.

RESULTS During a median follow-up of 14.1 years, 5,468 incident HF cases were documented. Total choline and phosphatidylcholine were positively associated with HF risk (HR: 1.08 [95% CI: 1.04-1.12] and HR: 1.08 [95% CI: 1.05-1.12], per one SD increase, respectively). Compared with the lowest quartile group, the HR for the highest quartile group was 1.23 (95% CI: 1.12-1.35) for total choline and 1.23 (95% CI: 1.12-1.34) for phosphatidylcholine. Glycine was inversely associated with HF risk (HR: 0.97 [95% CI: 0.94-0.99], per one SD increase). Participants with high polygenic risk score and high total choline or phosphatidylcholine had the highest risk of HF, whereas participants with low polygenic risk score and high glycine had the lowest risk. No statistically significant interactions were observed between choline metabolites and genetic susceptibility to HF. The Mendelian randomization analysis supported the potential causal associations of total choline (OR: 1.71 [95% CI: 1.01-1.35]) and glycine (OR: 0.93 [95% CI: 0.88-0.99]) with HF.

CONCLUSIONS Circulating choline metabolites were associated with the risk of incident HF, independent of genetic susceptibility. Whether targeting the metabolic pathway of choline might be a potential strategy for improving heart health warrants further validation. (JACC Adv. 2025;4:101445) © 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

BMI = body mass index

CRP = C-reactive protein

CVD = cardiovascular disease

eGFR = estimated glomerular filtration rate

GRS = genetic risk score

GWAS = genome-wide association studies

HF = heart failure

LDL = low-density lipoprotein

MR = Mendelian randomization

NMR = nuclear magnetic resonance

RCS = restricted cubic splines

SNP = single-nucleotide polymorphisms

TG = triglycerides

Heart failure (HF) is a severe and common outcome of various initial cardiac injuries that affects over 64 million people globally.[1] Patients with HF might experience multiple debilitating symptoms including dyspnea, fatigue, and limited exercise activity, causing poor quality of life for patients and substantial health care burden to society.[2,3] Despite considerable advances in HF treatment, effective ways for reversing and curing HF are still limited.[4] Thus, early identification of modifiable risk factors to prevent or postpone the development of HF is of profound clinical and public health significance.

Choline plays a crucial role in human metabolism, including the synthesis of phospholipids, betaine, and acetylcholine, all essential for cellular function and signaling.[5] In recent years, the potential role of choline and its metabolites in cardiovascular disease (CVD) has gained attention. Several studies have reported that elevated circulating levels of choline increase the risk of CVD in the general population.[6,7] However, the association of choline with incident HF remains unclear. To our knowledge, only a nest case-control study reported a positive association between circulating choline and HF.[8] However, the older population in this study (mean age: 70 years) may suffer a higher baseline risk of HF; also, the study had a relatively small sample size (n = 752). Therefore, the longitudinal evidence in this regard is inconclusive.

In addition, growing evidence has confirmed that genetic susceptibility might interact with metabolic factors to affect cardiometabolic outcomes,[9,10] but whether genetic susceptibility could modify the association between choline metabolites and HF risk is largely unknown. To fill the knowledge gaps, we aimed to examine the longitudinal association of choline metabolites (total choline, phosphatidylcholine, and glycine) with the development of HF using data from the UK Biobank. We also explored the possible joint or interactive effects of genetic risk and choline metabolites on HF risk. Finally, we applied 2-sample Mendelian randomization (MR) analyses to investigate the potential causal associations between circulating choline metabolites and HF.

METHODS

STUDY DESIGN AND PARTICIPANTS. The UK Biobank is a nationwide prospective cohort that recruited more than 500,000 participants aged 40 to 70 years through 22 assessment centers across England, Scotland, and Wales between 2006 and 2010. Details of the study design have been previously described.[11] Participants were asked to complete a touch screen questionnaire, record physical measurements, and provide biological samples. The UK Biobank study was approved by the North West Multi-Centre Research Ethics Committee and all participants provided written informed consent.

Among a total of 502,417 UK Biobank participants, 228,660 individuals were excluded because of missing data on choline metabolites. We further excluded 28,685 individuals with prevalent CVD (coronary heart disease, stroke, valve disease, atrial fibrillation, or HF), leaving 245,072 participants for final analysis (Supplemental Figure 1).

CHOLINE METABOLITES. Between April 2020 and June 2022, metabolic biomarkers were measured from 275,000 baseline ethylenediaminetetraacetic acid plasma samples using a high-throughput nuclear magnetic resonance (NMR) metabolomics platform developed by Nightingale Health Ltd. Briefly, the plasma samples were stored at −80 °C before preparation, were slowly thawed at +4 °C overnight, and were centrifuged (3,400g) for 3 minutes. Aliquots of all samples were transferred to NMR tubes and mixed with buffer. A 500 MHz proton NMR spectrometer (Bruker AVANCE IIIHD) and Nightingale Health's proprietary software were used to measure and quantify 3 choline metabolites including total choline, phosphatidylcholine, and glycine. Further details of the experiment can be found in the UK Biobank study document (https://biobank.ndph.ox.ac.uk/showcase/ukb/docs/NMR_companion_phase2.pdf).

ASCERTAINMENT OF HF. In the current study, the outcome of interest was incident HF. The diagnosis of HF was obtained by linkage to death register records, hospital inpatients, and primary care records. HF was defined based on the International Classification of Diseases-10th edition code I50.

GENETIC RISK SCORE FOR HF. Detailed information about genotyping, imputation, and quality control in the UK Biobank study has been described previously.[12] We created a genetic risk score (GRS) for HF using 12 single-nucleotide polymorphisms (SNPs) based on a previous genome-wide association study in European-descent participants (Supplemental Table 1).[13] Each SNP was recorded as 0, 1, or 2 according to the number of risk alleles; and then multiplied by the risk estimate (b coefficient) on HF obtained from the previous study to calculate the GRS: GRS = ($\beta 1 \times$ SNP1 + $\beta 2 \times$ SNP2 +. . .+$\beta 12 \times$ SNP12) $\times$ (12/sum of the β coefficients). We classified participants into 3 groups: low (tertile 1), medium

(tertile 2), and high (tertile 3) genetic risk of HF. Given the genetic difference between ethnicities, participants of non-European ancestry were excluded from the genetic analysis.

MEASUREMENTS OF COVARIATES. Self-reported information was collected by a touch screen questionnaire, including age, sex, race, the Townsend deprivation index, history of hypertension, diabetes, and chronic kidney disease, fasting time, physical activity, smoking status, moderate drinking (alcohol intake >0 g and ≤14 g/day for women and alcohol intake >0 g and ≤28 g/day for men[14]), diet score, and medication use (antidiabetic, antihypertensive, and lipid-lowering medication). The Townsend deprivation index is a composite measure of deprivation based on unemployment, noncar ownership, nonhome ownership, and household overcrowding. Townsend levels were stratified into high and low groups using the median value of the Townsend deprivation index. A higher Townsend deprivation index value indicates a lower socioeconomic level. The Metabolic Equivalent Task minutes based on items from a short International Physical Activity Questionnaire was adopted to assess physical activity. Since the variable 'ideal physical activity' was not used in the analysis, please remove this definition. For diet score, we used a definition of the ideal intake of dietary components for cardiovascular health,[15] as described in Supplemental Table 2. Each one point was given for each favorable diet factor, and the healthy diet score ranged from 0 to 10. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Biochemistry markers including glycated hemoglobin A1c (HbA1c), triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), C-reactive protein (CRP), and cystatin C were measured in the blood sample collected at recruitment. The estimated glomerular filtration rate (eGFR) was calculated via the 2021 Creatinine-Cystatin C EPI-CKD equation.[16]

STATISTICAL ANALYSES. Baseline characteristics of the analytic sample were summarized as numbers (percentages) for categorical variables and median (IQR) for continuous variables. The *t*-test and chi-square test were used to compare the characteristics by HF for continuous and categorical variables, respectively. Cox proportional hazards models were used to estimate the HRs and 95% CIs for incident HF associated with choline, phosphatidylcholine, and glycine. Follow-up time was calculated from the baseline date to the diagnosis of HF, death, or the censoring date (March 30, 2023), whichever came first. To reduce skewness, choline, phosphatidylcholine, and glycine were log-transformed; phosphatidylcholine and glycine were increased by 1 before log transformation due to certain values of 0. Choline metabolites were analyzed both as continuous variables and as categorical variables (Q1, Q2, Q3, Q4) based on quartiles. Model 1 was adjusted for: sex (male/female), age (years, continuous), race (White/others), education (university or college degree/others), Townsend deprivation index (continuous), BMI (kg/m^2, continuous), TG (mg/dL, continuous), LDL (mg/dL, continuous), fasting time (hours, continuous), smoking status (never, previous, current, or missing), moderate alcohol (yes/no), physical activity (minutes/week, continuous), and diet score (continuous). Model 2 was further adjusted for HF GRS (continuous) and eGFR (mL/min, continuous). Model 3 additionally included prevalent diabetes (yes/no), prevalent hypertension (yes/no), prevalent chronic kidney disease (yes/no), antidiabetes medication (yes/no), cholesterol-lowering medication (yes/no), and antihypertension medication (yes/no). If covariate information was missing, we imputed mean values for continuous variables and used a missing indicator approach for categorical variables. Restricted cubic splines (RCS) were used to assess the nonlinear association between choline metabolites and HF in the entire population, and stratified by sex and age. The RCS models were set with 4 knots at the 5th, 35th, 65th, and 95th centiles, with the median levels of metabolites as the reference point. Moreover, subgroup analyses were conducted stratified by sex (men/women), age (<65/≥65 years), BMI (<25/≥25 kg/m^2), TG (<2.26/≥2.26 mg/dL), eGFR (<60, 60-89, ≥90 mL/min), Townsend level (high/low), smoking status (never, former, or current), diabetes (yes/no), and hypertension (yes/no). The interactions were evaluated by introducing a product term between choline metabolites and stratification variables.

To examine the joint association between choline metabolites and HF GRS, we treated participants with low levels of choline metabolites and low GRS as the reference group. Both multiplicative and additive interaction analyses were performed to investigate whether genetic susceptibility modified the association between choline metabolites and HF risk. The multiplicative interaction was assessed by adding the product terms to the models. The additive interaction was evaluated by calculating the relative excess risk due to interaction and the attributable proportion due to interaction (AP).

Furthermore, we applied 2-sample MR to estimate the effect of choline metabolites on HF. The full

genome-wide association studies (GWAS) summary statistics of the 3 choline metabolites were made publicly available via the GWAS Catalog database with GWAS identifiers GCST90200327, GCST90132741, and GCST90269559. GWAS summary data for HF were from the FinnGen study (Supplemental Table 3). Causal estimates were calculated using the inverse-variance weighted method and several supplementary methods (MR-Egger, weighted median, weighted mode, and MR-PRESSO). Egger intercept, the global test for MR-PRESSO, and Steiger filtering analyses were applied to identify potential violations of MR assumptions.

We applied multivariable linear regression analyses to examine associations of choline metabolites with lipids (HDL, LDL-C, TG), BMI, HbA1c, eGFR, systolic blood pressure (SBP), and CRP. Models were adjusted for sex, age, race, education, Townsend deprivation index, smoking status, moderate alcohol, physical activity, diet score, BMI, HF GRS, eGFR, diabetes, chronic kidney disease, and hypertension. Cholesterol-lowering medication or antidiabetic medication were also included as confounders for lipids or HbA1c, respectively. Skewed variables were log-transformed for normal distribution. In addition, a correlation (Spearman) matrix of the choline metabolites and risk factors for HF was calculated.

Several sensitivity analyses were conducted to assess the association of choline metabolites with incident HF. To reduce potential reverse causation in observational analysis, we excluded participants who developed HF within the first 2 years of follow-up. Proportional subdistribution hazards regression models were used to account for competing risk of death. Missing values for covariates were imputed by multiple imputation with chained equations. We also analyzed the associations of baseline choline metabolite levels with the risk of incident HF within 1 to 5 years and beyond 5 years, for understanding of the temporal relationship between choline metabolites and HF risk during follow-up. Statistical significance was assessed at a threshold of $P < 0.05$. All analyses were performed in R, version 4.2.2. To address multiple testing, we further applied a more stringent threshold of $P < 0.0167$ (0.05/3) with Bonferroni correction to control false discovery rate.

RESULTS

BASELINE CHARACTERISTICS. During a median follow-up of 14.1 (IQR: 13.3-14.8 years, 3.4 million person-years), a total of 5,468 incident HF cases were observed. The baseline characteristics of the participants according to incident HF are shown in **Table 1**. Participants who had incident HF were older, mainly males, and had higher Townsend Deprivation index and higher prevalence of pre-existing diabetes, chronic kidney disease as well as hypertension compared with those without incident HF. In addition, they were more likely to be current smokers and had less physical activity. They also tended to have higher levels of BMI, TG, and CRP. For all choline metabolites, there was a higher percentage of females at higher, relative to lower, quartiles, as compared with males (all $P < 0.001$). Higher choline and phosphatidylcholine levels were associated with older age, higher socioeconomic status, higher levels of TG, LDL, CRP, and HF GRS. In contrast, higher glycine level was associated with younger age, lower BMI, TG, LDL, and CRP (Supplemental Tables 4 to 6).

PLASMA CHOLINE METABOLITES AND RISK OF HF. The associations between individual choline metabolites and HF are shown in **Table 2**. The risk of HF significantly increased per 1 SD increment in choline and phosphatidylcholine levels (HR: 1.08 [95% CI: 1.04-1.12] and HR: 1.08 [95% CI: 1.05-1.12], respectively). Conversely, glycine was inversely related to HF risk, with an HR of 0.97 (95% CI: 0.94-0.99) per 1 SD increment. Using RCS, we observed positive, nonlinear associations between both choline ($P_{\text{nonlinear}} = 0.041$) and phosphatidylcholine ($P_{\text{nonlinear}} = 0.012$) and HF risk in (**Figure 1**), particularly in men (Supplemental Figure 3). When dividing choline metabolite levels into quartiles, the HRs of HF associated with the highest quartiles of choline metabolites were 1.23 (95% CI: 1.12-1.35) ($P_{\text{trend}} < 0.001$ across quartiles) for total choline, 1.23 (95% CI: 1.12-1.34) ($P_{\text{trend}} < 0.001$) for phosphatidylcholine, and 0.93 (95% CI: 0.85-1.01) ($P_{\text{trend}} = 0.057$) for glycine, compared to the lowest quartile. These results remained stable after Bonferroni correction. The associations of choline metabolites with HF remained unchanged in serial sensitivity analyses when participants who developed HF during the first 2 years of follow-up were excluded, missing covariates were imputed, and competing risk of death was taken into account (Supplemental Tables 7 to 9). Our further analysis revealed that choline metabolites were not associated with HF risk within the first 5 years. However, they showed a significant association with HF risk beyond 5 years (Supplemental Table 10). Subgroup analysis showed significant interactions between sex and total choline or phosphatidylcholine on HF risk, with an increased risk observed in male but not in female. Similarly, interactions were found between hypertension and these metabolites (both

TABLE 1 Baseline Characteristics of the Study Population

	Overall (N = 245,072)	No HF (n = 239,604)	New-Onset HF (n = 5,468)	*P* Value
Female	137,715 (56.2)	135,374 (56.5)	2,341 (42.8)	<0.001
Age, y	57 (49-63)	57 (49-63)	63 (59-67)	<0.001
Ethnicity, White	231,944 (94.6)	226,720 (94.6)	5,224 (95.5)	0.012
University or college degree	80,135 (32.7)	78,962 (33.0)	1,173 (21.5)	<0.001
Townsend deprivation index	−2.24 (−3.70 to 0.34)	−2.25 (−3.70 to 0.32)	−1.77 (−3.41 to 1.30)	<0.001
Smoking status				<0.001
Never	136,746 (55.8)	134,431 (56.1)	2,315 (42.3)	
Former	82,123 (33.5)	79,864 (33.3)	2,259 (41.3)	
Current	25,071 (10.2)	24,217 (10.1)	854 (15.6)	
Missing	1,132 (0.5)	1,092 (0.5)	40 (0.7)	
Moderate alcohol drinking	77,365 (31.6)	75,751 (31.6)	1,614 (29.5)	<0.001
Fasting time, hours	3 (2-4)	3 (2-4)	4 (3-5)	<0.001
Physical activity, METs	1,788 (1,017-3,026)	1,788 (1,017-3,033)	1,788 (942-2,772)	<0.001
Diet score	3.00 (2.00-4.00)	3.00 (2.00-4.00)	3.00 (2.00-4.00)	<0.001
BMI, kg/m^2	26.61 (24.06-29.69)	26.58 (24.03-29.64)	28.67 (25.63-32.81)	<0.001
TG, mg/dL	129.80 (93.47-184.20)	129.80 (93.21-183.84)	139.63 (103.57-201.92)	<0.001
LDL, mg/dL	137.47 (117.33-158.78)	137.47 (117.53-158.89)	132.89 (107.89-153.63)	<0.001
HF GRS	8.38 (7.36-9.27)	8.38 (7.36-9.27)	8.38 (7.48-9.39)	<0.001
CRP, mg/L	1.30 (0.67-2.60)	1.30 (0.67-2.56)	1.90 (1.05-4.10)	<0.001
eGFR, mL/min/1.73 m^2	96.93 (87.42-105.66)	96.93 (87.66-105.80)	88.66 (77.39-97.27)	<0.001
Diabetes	10,975 (4.5)	10,253 (4.3)	722 (13.2)	<0.001
Chronic kidney disease	2,907 (1.2)	2,716 (1.1)	191 (3.5)	<0.001
Hypertension	62,655 (25.6)	59,934 (25.0)	2,721 (49.8)	<0.001
Medication for diabetes, cholesterol, or BP				
Antidiabetes	7,286 (3.0)	6,724 (2.8)	562 (10.2)	<0.001
Cholesterol lowering	32,461 (13.2)	30,843 (12.9)	1,618 (29.6)	<0.001
Antihypertension	42,462 (17.3)	40,263 (16.8)	2,199 (40.2)	<0.001
Choline, mmol/L	2.58 (2.32-2.85)	2.58 (2.32-2.85)	2.52 (2.23-2.81)	<0.001
Phosphatidylcholine, mmol/L	2.10 (1.87-2.36)	2.11 (1.87-2.36)	2.05 (1.80-2.32)	<0.001
Glycine, mmol/L	0.16 (0.13-0.20)	0.16 (0.13-0.20)	0.15 (0.12-0.19)	<0.001

Values are n (%) or median (IQR).

BMI = body mass index; BP = blood pressure; CRP = C-reactive protein; eGFR = estimated glomerular filtration rate; HF = heart failure; HF GRS = heart failure genetic risk score; LDL = low-density lipoprotein; MET = Metabolic Equivalent Task; TG = triglyceride.

$P_{interaction} < 0.001$), with the increased HF risk confined to participants with hypertension. Furthermore, participants with high TG levels exhibited a more pronounced increased HF risk associated with choline and phosphatidylcholine compared to those with low TG levels. For glycine, a stronger association with reduced HF risk was observed in individuals younger than 65 years, and a significant interaction was found between eGFR and glycine (Supplemental Figure 4).

JOINT ASSOCIATIONS OF CHOLINE METABOLITES AND HF GRS WITH INCIDENT HF. As expected, participants with medium and high GRS had a higher risk for HF than those with low GRS (HR: 1.11 [95% CI: 1.03-1.19] and 1.19 [95% CI: 1.11-1.27], respectively) (Supplemental Table 11). In the joint analyses, we found that participants with high genetic risk and Q4 choline levels had the highest HF risk, showing a 41% higher risk of HF (HR: 1.41 [95% CI: 1.22-1.63]) than participants with low genetic risk and choline levels ($P_{trend} < 0.001$) (**Figure 2**). A similar pattern was observed for phosphatidylcholine (HR: 1.39 [95% CI: 1.21-1.61]) ($P_{trend} < 0.001$). For glycine, a substantially lower risk of HF was observed in participants with low genetic risk and high glycine levels (Q4) ($P_{trend} < 0.001$). We did not observe a significant multiplicative interaction between the HF GRS and choline ($P_{interaction} = 0.700$), phosphatidylcholine ($P_{interaction} = 0.671$), or glycine ($P_{interaction} = 0.345$). Similarly, there were no additive interactions between HF GRS and these metabolites (Supplemental Table 12).

MR ANALYSIS TO ESTIMATE THE EFFECTS OF CHOLINE METABOLITES ON HF. In MR analysis, we found that choline was positively associated with HF risk (OR: 1.71, 95% CI: 1.01-1.35, $P = 0.031$),

TABLE 2 Associations of Baseline Individual Choline Metabolites Levels With the Risk of Incident Heart Failure

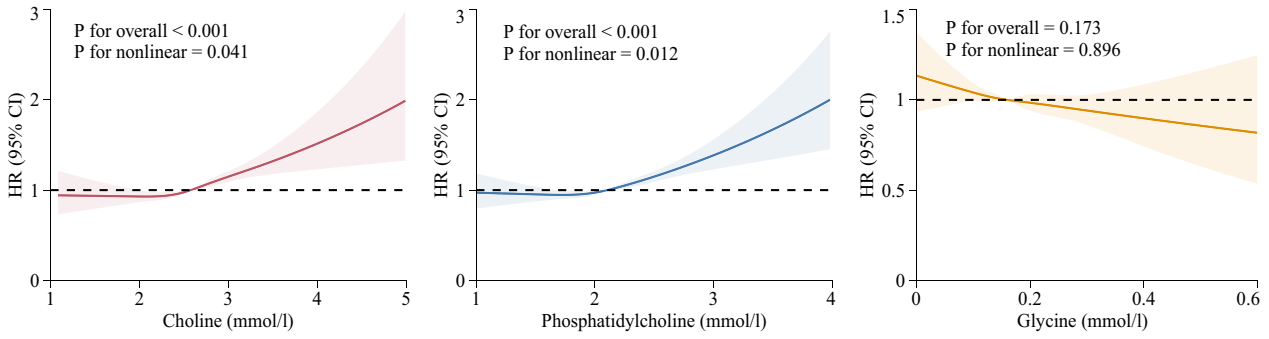
	Model 1		Model 2		Model 3	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Choline						
Per 1 SD increment	1.04 (1.00-1.07)	0.040	1.07 (1.04-1.11)	<0.001	1.08 (1.04-1.12)	<0.001
Q1	Ref		Ref		Ref	
Q2	0.96 (0.89-1.04)	0.319	1.00 (0.93-1.08)	0.915	1.03 (0.96-1.12)	0.393
Q3	1.04 (0.96-1.13)	0.331	1.10 (1.02-1.20)	0.017	1.13 (1.04-1.23)	0.004
Q4	1.13 (1.03-1.23)	0.011	1.23 (1.12-1.35)	<0.001	1.23 (1.12-1.35)	<0.001
P_{trend}	1.04 (1.01-1.07)	0.006	1.07 (1.04-1.10)	<0.001	1.07 (1.04-1.11)	<0.001
Phosphatidylcholine						
Per 1 SD increment	1.04 (1.00-1.07)	0.035	1.08 (1.04-1.11)	<0.001	1.08 (1.05-1.12)	<0.001
Q1	Ref		Ref		Ref	
Q2	0.96 (0.89-1.04)	0.333	1.01 (0.93-1.08)	0.883	1.03 (0.96-1.11)	0.403
Q3	1.02 (0.94-1.11)	0.579	1.09 (1.00-1.18)	0.039	1.11 (1.02-1.21)	0.012
Q4	1.12 (1.02-1.22)	0.015	1.22 (1.11-1.34)	<0.001	1.23 (1.12-1.34)	<0.001
P_{trend}	1.04 (1.01-1.07)	0.012	1.07 (1.04-1.10)	<0.001	1.07 (1.04-1.10)	<0.001
Glycine						
Per 1 SD increment	0.99 (0.95-1.02)	0.356	0.96 (0.93-0.99)	0.007	0.97 (0.94-0.99)	0.039
Q1	Ref		Ref		Ref	
Q2	0.98 (0.91-1.05)	0.557	0.95 (0.89-1.02)	0.184	0.97 (0.90-1.04)	0.336
Q3	0.97 (0.91-1.05)	0.492	0.93 (0.86-1.00)	0.047	0.94 (0.88-1.02)	0.134
Q4	0.97 (0.89-1.06)	0.477	0.90 (0.83-0.98)	0.020	0.93 (0.85-1.01)	0.081
P_{trend}	0.99 (0.96-1.02)	0.436	0.97 (0.94-0.99)	0.011	0.97 (0.95-1.00)	0.057

Choline raw value was log-transformed. For phosphatidylcholine and glycine, 1 was added to raw values before log transformation. Model 1: sex, age, race, education, Townsend deprivation index, BMI, TG, LDL, fasting time, smoking status, moderate alcohol drinking, physical activity, and diet score. Model 2: Model 1 plus HF GRS, and eGFR. Model 3: Model 2 plus diabetes, hypertension, chronic kidney disease, antidiabetes medication, cholesterol-lowering medication, and antihypertension medication.

Q = quartile; other abbreviations as in Table 1.

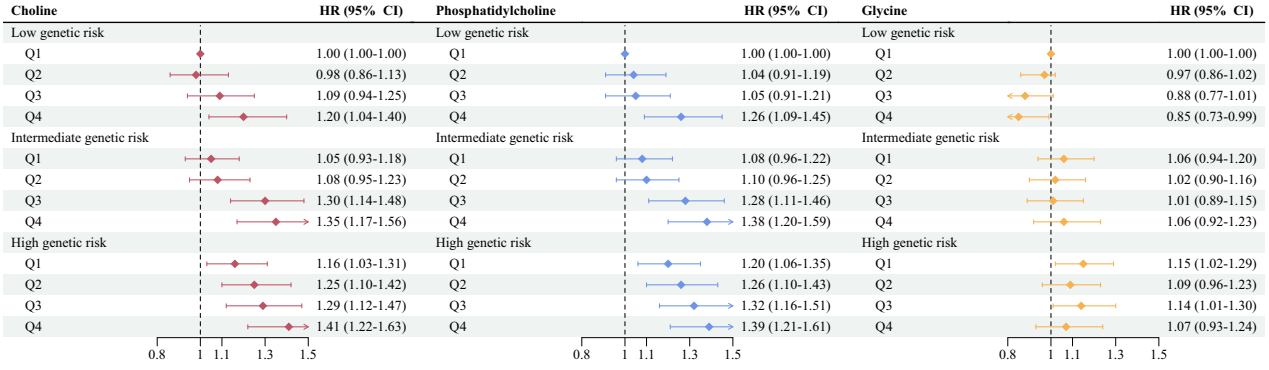
whereas glycine was inversely associated with HF (OR: 0.93, 95% CI: 0.88-0.99, $P = 0.026$) (Table 3). No significant association between phosphatidylcholine and HF risk was observed (OR: 1.12, 95% CI: 0.98-1.28, $P = 0.091$). There was little evidence of heterogeneity or horizontal pleiotropy, and no invalid SNPs were detected by the MR-Steiger filtering method (Supplemental Table 13).

FIGURE 1 Dose-Response Curves for Choline Metabolites and Incidence of Heart Failure



Multivariable model was adjusted for sex, age, race, education, Townsend deprivation index, fasting time, smoking status, moderate alcohol drinking, physical activity, diet score, BMI, TG, LDL, HF GRS, eGFR, diabetes, hypertension, chronic kidney disease, antidiabetes medication, cholesterol-lowering medication, and antihypertension medication. BMI = body mass index; eGFR = estimated glomerular filtration rate; HF GRS = heart failure genetic risk score; LDL = low-density lipoprotein; TG = triglyceride.

FIGURE 2 The Joint Association of Genetic Risk and Choline Metabolites With Heart Failure Among 231,944 European Ancestry Participants



Multivariable model was adjusted for sex, age, race, education, Townsend deprivation index, fasting time, smoking status, moderate alcohol drinking, physical activity, diet score, BMI, TG, LDL, HF GRS, eGFR, diabetes, hypertension, chronic kidney disease, antidiabetes medication, cholesterol-lowering medication, and antihypertension medication. Q = quartile; other abbreviations as in Figure 1.

CHOLINE METABOLITES IN RELATION TO LIPIDS, BMI, SBP, HbA1c AND CRP. In the multivariate linear regression analysis, choline and phosphatidylcholine were found to be positively correlated with HDL, LDL, BMI, TG, and SBP (all $P < 0.001$), while glycine was negatively correlated with HDL, LDL, BMI, TG, and SBP (all $P < 0.001$) (Supplemental Figure 5). Among the choline metabolites, choline was strongly correlated with phosphatidylcholine (r = 0.99), whereas glycine showed a weak correlation with choline and phosphatidylcholine (both r = 0.10) (Supplemental Figure 6).

DISCUSSION

In this large-scale cohort with a 14-year follow-up, we found that plasma total choline and phosphatidylcholine levels were significantly associated with an increased risk of HF, and the associations were not modified by genetic susceptibility to HF. MR analysis

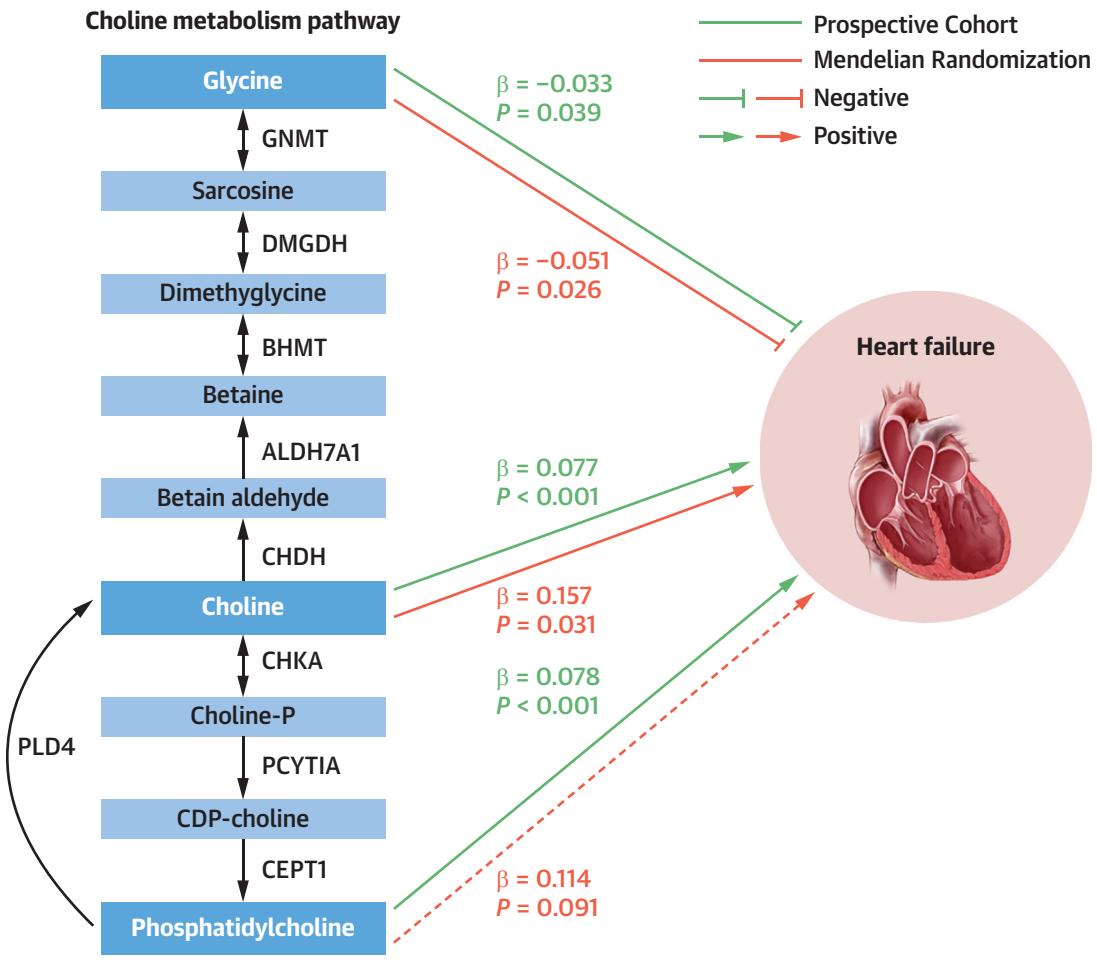
TABLE 3 MR Estimates of the Effect of Choline Metabolites on HF

Exposure	Outcome	Method	OR (95% CI)	*P* Value	Q Statistic	P_Q	Egger Intercept	$P_{intercept}$
Choline	HF	IVW	1.17 (1.01-1.35)	0.031	2.357	0.502		
		MR-Egger	1.01 (0.69-1.48)	0.972	1.677	0.432	0.017	0.496
		Weighted median	1.09 (0.92-1.31)	0.304				
		Weighted mode	1.09 (0.89-1.34)	0.474				
		MR-PRESSO	1.17 (1.03-1.32)	0.093	4.370	0.538		
Phosphatidylcholine	HF	IVW	1.12 (0.98-1.28)	0.091	12.489	0.052		
		MR-Egger	1.19 (0.85-1.69)	0.353	12.817	0.077	−0.008	0.705
		Weighted median	1.12 (0.98-1.27)	0.090				
		Weighted mode	1.12 (0.97-1.28)	0.136				
		MR-PRESSO	1.12 (0.98-1.28)	0.135	15.002	0.143		
Glycine	HF	IVW	0.93 (0.88-0.99)	0.026	28.905	0.067		
		MR-Egger	0.95 (0.88-1.02)	0.153	28.273	0.058	−0.004	0.534
		Weighted median	0.94 (0.90-0.99)	0.021				
		Weighted mode	0.95 (0.90-1.00)	0.053				
		MR-PRESSO	0.93 (0.88-0.99)	0.039	34.864	0.300		

OR, 95% CI, and *P* values were calculated for the respective method of MR analysis. The heterogeneity test in the IVW methods was performed using Cochran's Q statistic and the global test for the MR-PRESSO method. $P < 0.05$ was considered significant.

IVW = inverse-variance weighted; P_Q = *P* value for heterogeneity; $P_{intercept}$ = *P* value for the intercept of MR-Egger regression; other abbreviation as in Table 1.

CENTRAL ILLUSTRATION **Choline Metabolites, Genetic Susceptibility, and Incident Heart Failure**



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In choline metabolism pathway, single-side arrows indicate nonreversible reactions and double-side arrows indicate reversible reactions. In causal pathways, the arrows and T-shaped lines labeled signify positive and negative associations, respectively, in which the solid lines indicate significant causality and dotted lines nonsignificant. The β-values are the MR/Prospective estimates. ALDH7A1 = aldehyde dehydrogenase 7 family member A1; BHMT = betaine-homocysteine S-methyltransferase; CEPT1 = choline/ethanolamine phosphotransferase 1; CHDH = choline dehydrogenase; CHKA = choline kinase a; DMGDH = dimethylglycine dehydrogenase; GNMT = glycine N-methyltransferase; PCYT1A = phosphate cytidylyltransferase 1A; PLD4 = phospholipase D family member 4.

confirmed the causal association between choline and glycine and HF (Central Illustration). Participants with high genetic risk and high choline levels had the highest HF risk.

Although choline is an essential dietary nutrient that plays a wide range of physiological roles in human health, previous findings were conflicting regarding the association of choline and its metabolites with heart disease. Some basic studies suggested that choline exhibited cardioprotective effects against several heart diseases including myocardial infarction, ischemia/reperfusion injury, and cardiac hypertrophy.[17,18] On the other hand, it was found that plasma choline was elevated in patients with chronic HF.[19] In a recent nest case-control study within the PREDIMED cohort, it was observed that baseline plasma choline was independently associated with an increased risk of HF.[8] However, the participants were older Mediterranean individuals with a mean age of 70 years and the sample size was relatively small (n = 752), which limited the generalizability of the results. Our study extends this finding to the middle-

aged population using data from a large population-based study. Furthermore, we explored the interaction between choline metabolites and genetic susceptibility to HF and found little evidence of the effect modification by genetic susceptibility. Several mechanisms might explain the association between choline and HF. It was reported that choline could induce inflammation by NLRP3 inflammasome activation and IL-1β production,[20] thereby exacerbating the pathological process of HF. Choline could also reduce the overload of intracellular Ca^{2+} in isolated myocytes, which in turn inhibits the activation of the AKT pathway,[21] further weakening the heart's protective mechanisms.[22] Moreover, we observed choline was associated with higher SBP, LDL, BMI, and TG levels, which were well-established risk factors for HF. Our MR analysis further suggested the potential causal association between choline and HF.

Our prospective study also examined the relationship between 2 choline metabolites, phosphatidylcholine and glycine, and the risk of HF. Similar to a previous study, we found that phosphatidylcholine was significantly associated with an increased risk of HF.[23] This might be because phosphatidylcholine is a key structural component of mammalian cell membranes, and its dysregulation can affect membrane integrity, disrupt myocardial metabolism and cell signaling,[24] and lead to adverse left ventricular remodeling.[25] For glycine, previous observational[26] and MR studies[27] showed a negative correlation with coronary heart disease. However, evidence on the association between glycine and HF is limited. Our study found that glycine showed a negative correlation with HF risk, and it was associated with lower BMI, SBP, TG, and CRP levels. In fact, glycine has been reported to lower blood pressure,[28] regulate lipid metabolism,[28] and prevent oxidative stress as a substrate for glutathione biosynthesis, a major antioxidant in cells,[29] which could explain the cardioprotective effects of glycine on HF. Furthermore, a recent MR study in East Asian populations supported a negative correlation between glycine and chronic HF,[30] and our MR analysis further confirmed the protective effect of glycine against HF.

In the joint association of choline metabolites and genetic susceptibility, we observed that high genetic risk and total choline or phosphatidylcholine conferred the highest risk of HF, even though the test on the interaction between the choline metabolites and genetic susceptibility was not significant. The proportion of HF risk explained by the variants was <10%, which may partially explain the negative interaction. Existing evidence suggests the potential mechanisms for the risk of HF associated with a higher choline and phosphatidylcholine strengthened by a high genetic risk. The identified genetic loci for HF in the genome-wide association study were associated with risk factors and traits related to left ventricular structure and function. The genetic loci associated with reduced left ventricular systolic function or atrial fibrillation were also related to the processes of cardiac development, protein homeostasis, and cellular senescence. In addition, the observed relations between choline, phosphatidylcholine, and HF might be through these aforementioned mechanical changes. Therefore, we assumed that choline metabolites and genetic variations for HF risk might have additive effects on the risk of HF through at least certain overlapped biological mechanisms related to cardiac function.

In stratified analyses, we observed a sex difference in the relationship between choline metabolites and risk of HF. Previous evidence suggested that choline intake was associated with lower body fat mass and waist-to-hip ratio in females,[31] which were found to decrease the risk of HF.[32,33] Therefore, the adverse effects of high choline levels on HF in females might not be as significant as observed in males. Furthermore, the risk of HF associated with choline and phosphatidylcholine was higher in participants with high TG levels than those with low TG levels. This may be related to the impact of choline and its metabolites on lipid metabolism.[34] Prior researches have shown that an increase in choline or a decrease in the betaine/choline ratio was associated with an increase in plasma TG levels,[35] consistent with our results. Notably, significant interactions were found between choline, phosphatidylcholine, and hypertension. Recent basic research also demonstrated that choline induced cardiac dysfunction by inhibiting the production of endogenous hydrogen sulfide in spontaneously hypertensive rats.[36] This finding aligns with our observation that high choline levels significantly increase the risk of HF in hypertensive individuals. Our study suggests that maintaining appropriate choline levels may be crucial for preventing HF in men with high TG levels or those with hypertension.

To the best of our knowledge, this is the first prospective study to assess the longitudinal associations of choline metabolites, genetic risk, and incident HF. The strengths of this study include its large sample size, long-term follow-up, prospective study design, and reliable genetic data. The MR analysis provided genetic evidence for the causal association between choline metabolites and HF.

STUDY LIMITATIONS. Several limitations also remained in our study. First, the definition of HF was

based on self-reports and medical records, which may lead to some missing cases, and there might be potential regional diagnostic differences. Additionally, there was a lack of left ventricular ejection fraction information, limiting the ability to evaluate HF with preserved or reduced ejection fractions. Second, repeated measurements of choline metabolites were not available and we were unable to evaluate the association between change or variability in choline metabolites levels and HF. Third, although we carefully adjusted for various potential confounders, bias from unknown or unmeasured confounding factors may still exist. Finally, the predominance of participants of European descent limits the generalizability of our findings, warranting external validation in other cohorts to confirm and strengthen our results.

CONCLUSIONS

In summary, our study demonstrated that circulating total choline and phosphatidylcholine were positively associated with the risk of HF, while circulating glycine was negatively associated with HF risk. The observed associations between choline metabolites and HF were not modified by genetic susceptibility. Further studies are needed to confirm our findings and investigate the underlying mechanisms.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Circulating total choline and phosphatidylcholine were positively associated with the risk of HF, while circulating glycine was negatively associated with HF risk, regardless of genetic susceptibility.

TRANSLATIONAL OUTLOOK: Circulating choline metabolites can serve as independent markers for incident HF. Targeting these metabolites for early intervention may reduce HF risk. Given the positive association of total choline and phosphatidylcholine with HF risk, lifestyle or pharmacological interventions aimed at reducing these metabolite levels could be beneficial. Conversely, increasing glycine levels through diet or supplementation might offer a protective effect against HF.

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KEY WORDS **choline metabolism, genetic risk, heart failure, Mendelian randomization, prospective cohort**

APPENDIX **For supplemental methods, tables, and figures, please see the online version of this paper.**