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Mediating role of the Dietary Index for Gut Microbiota in the association between depression and cardiovascular health among US adults

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Abstract

Background: Depression is closely associated with cardiovascular health, and gut microbiota–related dietary patterns may represent a potential biological pathway linking the two. This study aimed to examine the association between depressive symptoms and cardiovascular health as defined by Life's Essential 8 (LE8), and to evaluate the mediating role of the Dietary Index for Gut Microbiota (DI-GM) in this association.

Methods: The study included 18,071 adults aged ≥20 years from the US National Health and Nutrition Examination Survey (NHANES) 2007-2018. The primary outcome was LE8 cardiovascular health rating (low/moderate/high), with LE8 continuous total score as supportive outcome; depression was assessed by Patient Health Questionnaire (PHQ-9). Survey-weighted ordinal logistic regression and linear regression were used to assess the association between depression and DI-GM and LE8. Restricted cubic spline (RCS) was used to evaluate the dose-response relationship, and counterfactual (g-computation) mediation analysis was used to explore the mediating effect of DI-GM. Effect modification was also examined in subgroups. Models were adjusted for age, sex, race/ethnicity, marital status, education level, and poverty-to-income ratio (PIR).

Results: After adjusting for potential confounders, each 1-point increase in PHQ-9 was associated with a 9% reduction in the cumulative odds of having a higher LE8 score (OR=0.91,

95% CI: 0.90–0.93), whereas each 1-point increase in DI-GM was associated with a 46% increase in the cumulative odds of having a higher LE8 score (OR=1.46, 95% CI: 1.42–1.51) (both $P<0.001$). DI-GM partially mediated the association between depression and LE8, the total natural indirect effect was statistically significant (TNIE OR=0.996, 95% CI 0.993–0.999, $P=0.008$), accounting for 5.08% (95% CI 1.35–8.87%) of the total association, estimated within a counterfactual mediation framework. This mediating pathway was more pronounced among women (mediation proportion: 6.0%) and individuals with comorbid gastrointestinal diseases (14.2%). Dose–response analyses indicated that these associations generally followed a monotonic pattern.

Conclusions: A greater burden of depressive symptoms was associated with poorer cardiovascular health as assessed by LE8, suggesting that gut microbiota-related dietary patterns may partially mediate the association between depression and cardiovascular health. DI-GM may represent a potential modifiable target, particularly among women and individuals with comorbid gastrointestinal diseases. However, the temporal sequence and underlying mechanisms of these associations require further validation through longitudinal and intervention studies.

Keywords: Depression, LE8, Cardiovascular Health, DI-GM, NHANES

Introduction

Cardiovascular disease (CVD) remains the leading cause of death worldwide, with its disease burden continuing to increase[1]. In recent years, the paradigm of CVD prevention has shifted from the management of individual risk factors toward the promotion of overall cardiovascular health. Accordingly, the American Heart Association (AHA) introduced Life's Essential 8 (LE8) in 2022, a comprehensive metric that quantifies cardiovascular health across eight domains: diet, physical activity, nicotine exposure, sleep, body mass index, blood lipids, blood glucose, and blood pressure. LE8 provides a continuous score ranging from 0 to 100 and categorizes cardiovascular health into low, moderate, and high levels. Compared with the previous Life's Simple 7 (LS7), LE8 offers a more comprehensive assessment and greater clinical relevance[2]. This shift has also raised a question: which modifiable factors are associated with overall cardiovascular health at the population level?

Among the numerous factors influencing cardiovascular health, depression has attracted particular attention. Depression is one of the most prevalent mental disorders worldwide and a leading cause of disability[3,4], with its global burden having further increased in recent years owing to population aging and the coronavirus disease 2019 (COVID-19) pandemic[5], Accumulating evidence suggests a bidirectional relationship between depression and cardiovascular disease (CVD)[6]. Depression has been associated with the accelerated development of multiple cardiovascular risk factors[7], and the American Heart Association has issued a scientific statement highlighting the interconnections among the heart, brain, and body, while recommending routine assessment of psychological health in individuals at high cardiovascular risk[8]. However, previous studies have primarily focused on individual cardiovascular risk factors or clinical cardiovascular events as outcomes. Although analyses based on the National Health and Nutrition Examination Survey (NHANES) have reported an association between LE8 and depression[9,10], most have treated LE8 as the exposure and depression as the outcome. In contrast, relatively few studies have examined depression as the exposure in relation to overall cardiovascular health, and even fewer have explored the potential behavioral or biological mechanisms that may mediate this association.

If depression is indeed associated with overall cardiovascular health, an important subsequent question is through which pathways this association may operate. Among the candidate mechanisms, diet is of particular interest because it has a dual role: it is both a common behavioral manifestation of depression and a direct determinant of cardiovascular health, placing it conceptually at the intersection of these two conditions[11,12]. An important

intermediary linking diet and cardiovascular health is the gut microbiota. On the one hand, through the microbiota–gut–brain axis, the gut microbiota contributes to emotional regulation via immune, neuroendocrine, and vagal pathways, as well as microbial metabolites such as short-chain fatty acids[13]. Patients with depression commonly exhibit reduced gut microbial diversity and altered microbial composition[14–15]. On the other hand, through the gut–heart axis, the gut microbiota and its metabolites influence cardiovascular processes, including blood pressure regulation, lipid metabolism, and inflammation[16–18]. For example, trimethylamine N-oxide (TMAO), a microbial metabolite derived from dietary precursors abundant in foods such as red meat, has been associated with atherosclerosis and an increased risk of cardiovascular disease[19]. Taken together, the conceptual framework linking depression, gut microbiota–related dietary patterns, and cardiovascular health represents a biologically plausible and potentially modifiable pathway worthy of further investigation.

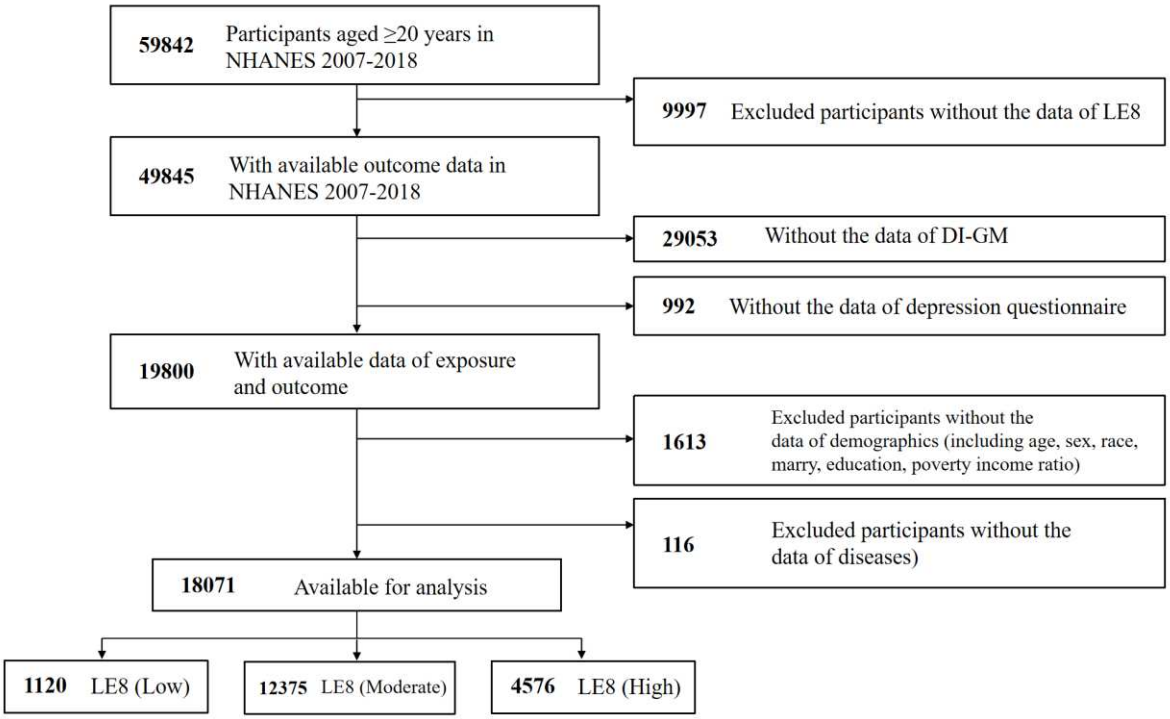
However, direct measurement of the gut microbiota is not feasible in large-scale population studies, making this pathway difficult to investigate at the epidemiological level. Diet is one of the most important modifiable determinants of gut microbiota composition and function[20,21]. To address this challenge, Kase et al. developed the Dietary Index for Gut Microbiota (DI-GM) based on existing evidence, which integrates foods considered beneficial or detrimental to the gut microbiota to characterize the gut microbiota–friendliness of overall dietary patterns[22]. By using dietary information as a proxy for gut microbiota, the DI-GM enables this pathway to be quantified in population-based studies without the need for microbiome sequencing. Recent analyses of NHANES data have shown that a higher DI-GM is associated with a lower risk of cardiovascular disease[23], as well as a lower risk of depression, with evidence suggesting a potential mediating mechanism[24]. However, these findings remain limited to pairwise associations. To our knowledge, no study has integrated all three factors within the same population to examine whether the DI-GM mediates the association between depression and overall cardiovascular health, as assessed by Life's Essential 8 (LE8).

Therefore, using nationally representative data from U.S. adults participating in the National Health and Nutrition Examination Survey (NHANES) 2007–2018, this study aimed to examine the association between depressive symptoms and cardiovascular health, as assessed by Life's Essential 8 (LE8), and to evaluate the potential mediating role of the Dietary Index for Gut Microbiota (DI-GM) in this association.

Methods

1. Study design and population

This study was a cross-sectional analysis based on six consecutive cycles of the National Health and Nutrition Examination Survey (NHANES) from 2007 to 2018. NHANES is conducted by the National Center for Health Statistics (NCHS) of the U.S. Centers for Disease Control and Prevention and uses a stratified, multistage probability sampling design to obtain a nationally representative sample. Data are collected through household interviews, physical examinations, and laboratory assessments.The NHANES protocols were approved by the NCHS Research Ethics Review Board, and written informed consent was obtained from all participants. Among the 59,842 participants initially identified, 9,997 were excluded because of missing data required for the calculation of Life's Essential 8 (LE8), 29,053 because of missing dietary data required for the Dietary Index for Gut Microbiota (DI-GM), 992 because of missing Patient Health Questionnaire-9 (PHQ-9) data, 1,613 because of missing sociodemographic data, and 116 because of missing disease-related information. The final analytic sample comprised 18,071 adults (Figure 1).



2. Assessment of depression (exposure)

Depression was assessed using the Patient Health Questionnaire-9 (PHQ-9). The PHQ-9 is based on the diagnostic criteria for depression in the Diagnostic and Statistical Manual of

Mental Disorders, Fifth Edition (DSM-5) and consists of nine items, each scored from 0 (not at all) to 3 (nearly every day), yielding a total score ranging from 0 to 27. A cutoff score of 10 has demonstrated good sensitivity and specificity for screening major depression[25,26]. In the present study, the PHQ-9 was analyzed both as a continuous variable (per 1-point increase in score) and as a binary variable, with scores ≥10 indicating depression and scores <10 indicating no depression.

3. Dietary Index for Gut Microbiota (mediator)

The Dietary Index for Gut Microbiota (DI-GM) was constructed according to the scoring criteria proposed by Kase et al. and comprises 14 food or nutrient components. Components considered beneficial to the gut microbiota included avocado, broccoli, chickpeas, coffee, cranberries, fermented dairy products, dietary fiber, soy, whole grains, and green tea, whereas red meat, processed meat, refined grains, and a high-fat diet (≥40% of total energy intake from fat) were considered unfavorable components. The DI-GM was calculated based on 24-hour dietary recall data. For beneficial components, 1 point was assigned when intake was at or above the sex-specific median and 0 points otherwise. For unfavorable components, 1 point was assigned when intake was below the sex-specific median; for the high-fat diet component, 1 point was assigned when fat contributed <40% of total energy intake. Otherwise, 0 points were assigned. The component scores were summed to obtain the total DI-GM score, ranging from 0 to 13 (Due to the lack of green tea data in NHANES, this information could not be obtained, 13 components were used, yielding a score of 0–13), including a beneficial-component score of 0–9 and an unfavorable-component score of 0–4. Participants were further categorized into four groups according to their total DI-GM scores (0–3, 4, 5, and ≥6). In addition, the beneficial-component and unfavorable-component scores were calculated separately[22].

4. Assessment of cardiovascular health (outcome)

Cardiovascular health was assessed using Life's Essential 8 (LE8), introduced by the American Heart Association (AHA) in 2022. LE8 comprises four health behaviors (diet, physical activity, nicotine exposure, and sleep) and four health factors (body mass index, blood lipids, blood glucose, and blood pressure). Each component is scored continuously on a scale from 0 to 100, and the overall LE8 score (0–100) is calculated as the unweighted mean of the eight component scores. According to the AHA criteria, cardiovascular health was categorized as low (LE8 score <50), moderate (50–79), and high (≥80)[2]. In the present

study, LE8 category was considered the primary outcome, while the continuous overall LE8 score was used as a supportive outcome.

5. Covariates

Based on previous evidence and clinical knowledge, the following potential confounders were included in the present study: age, sex, race/ethnicity, marital status, educational attainment, and the poverty-to-income ratio (PIR)[24]. In the regression models, age and PIR were included as continuous variables. For the description of baseline characteristics and subgroup analyses, age was categorized as 20–39, 40–59, and ≥60 years, while PIR was categorized as ≤1.30, 1.31–3.50, and >3.50. Race/ethnicity was classified as Mexican American, non-Hispanic White, non-Hispanic Black, other Hispanic, or other race/ethnicity. Marital status was dichotomized as married or living with a partner versus unmarried, and educational attainment was categorized as less than high school, high school or equivalent, and above high school.

6. Statistical analysis

All analyses accounted for the complex, multistage probability sampling design of NHANES by incorporating the appropriate survey weights, strata (SDMVSTRA), and primary sampling units (SDMVPSU) to obtain nationally representative estimates. Variance estimates were calculated under the complex survey design using Taylor series linearization. Because the DI-GM was derived from 24-hour dietary recall data, dietary interview sample weights were applied. A combined 12-year survey weight was constructed for the six NHANES cycles (2007–2018) included in the analysis. The primary analyses were based on complete-case analysis.

Participants were categorized into low (<50), moderate (50–79), and high (≥80) cardiovascular health groups according to their LE8 scores for the description of baseline characteristics. Continuous variables with an approximately normal distribution were presented as survey-weighted means with standard errors (SEs) and compared using design-based Wald tests. Skewed continuous variables were summarized as medians with interquartile ranges (IQRs). Categorical variables were presented as survey-weighted counts and percentages and compared using the Rao–Scott second-order corrected chi-square test. When the overall test was statistically significant, pairwise comparisons were performed with Bonferroni adjustment for multiple testing.

The primary objective of this study was to examine whether the Dietary Index for Gut Microbiota (DI-GM) mediates the association between depressive symptoms and cardiovascular health, as assessed by Life's Essential 8 (LE8). In the primary mediation analysis, the continuous PHQ-9 score was specified as the exposure, the DI-GM as the mediator, and LE8 cardiovascular health category (low, moderate, or high) as the outcome. Accordingly, LE8 category was defined as the primary outcome. Therefore, the mediation analysis was repeated using the continuous LE8 score as the outcome under a linear regression framework as a sensitivity analysis to evaluate the robustness of the findings (see the Supplementary Material).

As a prerequisite for mediation analysis, the associations required to establish mediation were first examined. When LE8 category was the outcome, survey-weighted ordinal logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs), with ORs >1 indicating a greater likelihood of belonging to a higher LE8 category. The proportional-odds assumption was assessed by fitting, at each cumulative cut point of the LE8 category (moderate or high versus low; high versus low or moderate), a separate survey-weighted binary logistic model with the same covariates, and testing equality of the coefficient vectors. The covariance matrix of the between-cut-point difference was estimated from 500 Rao–Wu rescaled bootstrap replicates that preserve the NHANES strata and primary sampling units, and a design-based Wald statistic was referred to an F distribution with the survey design degrees of freedom (df = 94). In addition to the global test over all model terms, the test was partitioned into the components attributable to the exposure of interest and to the covariates (Supplementary Table S7). When the continuous LE8 score was the outcome, survey-weighted linear regression was used to estimate regression coefficients (β) and 95% CIs. Depression was modeled both as a continuous variable (PHQ-9 score, per 1-point increase) and as a binary variable (PHQ-9 ≥10 vs <10). The DI-GM was analyzed as a continuous variable (per 1-point increase), as a categorical variable with four levels (0–3, 4, 5, and ≥6; 0–3 as the reference category). Tests for linear trend were performed by assigning each participant the median DI-GM value of his or her category (2, 4, 5 and 6, respectively) and entering this variable as a single continuous term in place of the categorical indicators; the *P* value for trend is the design-based Wald test of this term (1 degree of freedom), with variances estimated by Taylor series linearisation and the statistic referred to a t distribution with the survey design degrees of freedom (df = 94). The DI-GM was additionally analysed as separate beneficial- and unfavorable-component scores. For each association, both crude

and adjusted models were fitted. The adjusted models included age, sex, race/ethnicity, marital status, educational attainment, and the poverty-to-income ratio (PIR) as covariates.

To characterize the dose–response relationships underlying the mediation framework and provide graphical evidence for the hypothesized pathways, restricted cubic spline (RCS) analyses were performed to evaluate the associations between depression and LE8 (corresponding to the total effect pathway) and between the DI-GM and LE8 (corresponding to the mediator-to-outcome pathway, path *b*). Knots were placed at the 10th, 50th, and 90th percentiles of the exposure distribution. Overall associations were assessed using joint tests of the spline terms, whereas departures from linearity were evaluated using tests of the nonlinear spline components. Given the high statistical power of large samples to detect trivial nonlinearities, the spline curves were interpreted primarily according to their overall direction and shape.

Mediation by DI-GM was assessed within the counterfactual (potential-outcomes) framework rather than by the traditional product-of-coefficients approach, because the outcome model for LE8 category uses a logit link, under which the total effect is not the algebraic sum of the direct and indirect effects. A mediator model (exposure → DI-GM) was fitted by survey-weighted linear regression, and an outcome model (exposure, DI-GM, their interaction, and covariates → LE8) by survey-weighted cumulative logit regression for LE8 category or survey-weighted linear regression for the continuous LE8 score. Counterfactual quantities were identified by g-computation (the imputation estimator): the mediator distribution was represented by the weighted empirical distribution of the mediator-model residuals (40 equally weighted quantiles), so that no normality assumption was imposed, and the resulting potential-outcome distributions were standardised over the covariate distribution of the analytic sample using the survey weights. For the exposure contrast a versus a* (a 1-point increment in the PHQ-9 score, or depression defined as PHQ-9 ≥10 versus <10), we estimated the total effect (TE), the pure natural direct effect (PNDE), the total natural indirect effect (TNIE) and the controlled direct effect with DI-GM fixed at its weighted mean. Because the primary outcome is ordinal, effects are reported as marginal cumulative odds ratios derived from a marginal proportional-odds summary of the counterfactual distributions; cut-point-specific marginal odds ratios and effects on the probability (risk-difference) scale are provided in Supplementary Table S4. The primary effect was summarized by a marginal cumulative odds ratio, which remains a valid summary measure even when the proportional-

odds assumption is violated (cut-point-specific results are provided in Supplementary Table S4.1.) The proportion mediated was computed as OR_PNDE × (OR_TNIE − 1) / (OR_TE − 1) on the odds-ratio scale and as TNIE/TE on the natural scale, and the decomposition is exact on both scales. Identification of the natural direct and indirect effects requires no unmeasured confounding of the exposure–outcome, mediator–outcome or exposure–mediator relationships and no mediator–outcome confounder affected by the exposure. All 95% confidence intervals (CIs) and P values for counterfactual quantities are percentile intervals and design-based tests from 500 Rao–Wu rescaled bootstrap replicates that resample primary sampling units within strata, thereby preserving the NHANES complex survey design; the Sobel (delta) method was not used. To evaluate robustness, mediation analyses were repeated across four exposure–outcome combinations: continuous PHQ-9 score or depression status as the exposure, and LE8 category or continuous LE8 score as the outcome. Prespecified subgroup analyses were performed within the same framework by domain estimation according to age, sex, race/ethnicity, marital status, educational attainment, the poverty-to-income ratio (PIR) and major comorbidities; the variable defining each subgroup was not adjusted for within the corresponding subgroup analysis. In addition, joint associations of depression status and DI-GM with LE8 were evaluated using a 2 × 2 joint exposure classification (depression status × dichotomized DI-GM), and multiplicative interaction was assessed. All statistical analyses were performed using RStudio (version 4.2.3). All tests were two-sided, and $P < 0.05$ was considered statistically significant.

Results

1. Participant characteristics

After sequential exclusions, a total of 18,071 adults aged ≥20 years were included in the final analysis (Figure 1). According to LE8 scores, 1,120 participants had low (<50), 12,375 had moderate (50–79), and 4,576 had high (≥80) cardiovascular health. With the exception of marital status, baseline characteristics differed significantly across the three groups, including age, sex, race/ethnicity, educational attainment, poverty-to-income ratio (PIR), DI-GM score, PHQ-9 score, and the prevalence of depression (Table 1).Mean DI-GM scores increased progressively across LE8 categories (3.06, 3.49, and 4.37 for the low, moderate, and high LE8 groups, respectively), whereas mean PHQ-9 scores (5.41, 2.98, and 2.23, respectively) and the prevalence of depression (19.75%, 7.55%, and 3.84%, respectively) decreased with increasing cardiovascular health. Results of Pairwise comparisons between groups and the complete baseline characteristics are presented in Supplementary Table S1.

Table 1 Survey-weighted characteristics of NHANES 2007–2018 participants by LE8 category

Characteristics	Total	Low (<50)	Moderate (50–79)	High (≥80)	*P* value
Participants, n (unweighted)	18,071	1,120	12,375	4,576	
Weighted population, n (millions)	165.85	7.86	108.49	49.49	
Age, years, mean (SE)	45.40 (0.30)	48.69 (0.56)	47.45 (0.30)	40.37 (0.45)	<0.001
Sex (female), n (millions), %	79.79 (48.11)	3.35 (42.60)	46.71 (43.06)	29.73 (60.07)	<0.001
Race / ethnicity, n (millions), %					<0.001
Mexican American	13.68 (8.25)	0.54 (6.89)	9.62 (8.87)	3.52 (7.11)	
Other Hispanic	8.80 (5.31)	0.45 (5.72)	5.78 (5.33)	2.57 (5.19)	
Non-Hispanic White	114.69 (69.16)	4.60 (58.44)	74.16 (68.36)	35.94 (72.61)	
Non-Hispanic Black	16.05 (9.68)	1.68 (21.37)	11.55 (10.65)	2.82 (5.69)	
Other Race	12.63 (7.61)	0.60 (7.59)	7.37 (6.80)	4.65 (9.40)	
Married / living with partner, n (millions), %	104.62 (63.08)	4.56 (58.03)	68.88 (63.49)	31.18 (62.99)	0.091
Education level, n (millions), %					<0.001
Less than high school	20.05 (12.09)	1.81 (23.02)	15.34 (14.14)	2.91 (5.87)	
High school or equivalent	36.57 (22.05)	2.72 (34.58)	28.03 (25.84)	5.82 (11.76)	
Above high school	109.22 (65.86)	3.33 (42.40)	65.12 (60.02)	40.77 (82.37)	
PIR, mean (SE)	3.10 (0.04)	2.21 (0.09)	3.02 (0.04)	3.43 (0.06)	<0.001
DI-GM score, mean (SE)	3.73 (0.03)	3.06 (0.07)	3.49 (0.02)	4.37 (0.04)	<0.001
DI-GM category, n (millions), %					<0.001
0–3	74.32 (44.81)	4.94 (62.79)	55.12 (50.81)	14.26 (28.80)	
4	38.23 (23.05)	1.77 (22.49)	25.18 (23.21)	11.29 (22.80)	
5	29.61 (17.85)	0.82 (10.47)	17.28 (15.93)	11.51 (23.25)	

≥6	23.69 (14.28)	0.33 (4.25)	10.91 (10.05)	12.45 (25.15)	
PHQ-9 score, mean (SE)	2.87 (0.05)	5.41 (0.29)	2.98 (0.06)	2.23 (0.08)	<0.001
Depression (PHQ-9 ≥10), n (millions), %	11.64 (7.02)	1.55 (19.75)	8.19 (7.55)	1.90 (3.84)	<0.001
Diabetes, n (millions), %	12.35 (7.45)	2.08 (26.48)	9.90 (9.13)	0.37 (0.74)	<0.001
GI disease, yes, n (millions), %	11.04 (6.66)	1.05 (13.40)	7.33 (6.76)	2.66 (5.37)	<0.001
Sleep problem, yes, n (millions), %	45.00 (27.14)	3.71 (47.12)	31.22 (28.78)	10.07 (20.35)	<0.001
Stroke, yes, n (millions), %	3.14 (1.90)	0.40 (5.09)	2.35 (2.17)	0.39 (0.80)	<0.001
Cancer, yes, n (millions), %	15.51 (9.35)	0.60 (7.59)	11.11 (10.25)	3.80 (7.67)	<0.001

Continuous variables are presented as survey-weighted means (SEs), and categorical variables as weighted population estimates (millions) and weighted column percentages (%).
[a] Group differences were assessed using design-based tests: Wald tests for continuous variables and Rao–Scott corrected chi-square tests for categorical variables.
[b] All estimates accounted for the complex NHANES sampling design using the 12-year survey weights (Final_weight_12yr), strata (SDMVSTRA), and primary sampling units (SDMVPSU); the survey design degrees of freedom were 94. Baseline characteristics including all comorbidity variables are presented in Supplementary Table S1.
Abbreviations: DI-GM, Dietary Index for Gut Microbiota; GI, gastrointestinal; LE8, Life's Essential 8; PHQ-9, Patient Health Questionnaire-9; PIR, poverty-to-income ratio; SE, standard error.

2. Associations of depression and DI-GM with LE8, and their dose–response form

Depression was significantly associated with poorer cardiovascular health as assessed by LE8. In survey-weighted ordinal logistic regression models with LE8 category as the primary outcome, each 1-point increase in PHQ-9 score was associated with a 9% lower cumulative odds of being in a higher LE8 category (OR=0.91, 95% CI: 0.90–0.93), and participants with depression had significantly lower odds compared with those without depression (OR=0.39, 95% CI: 0.29–0.51; both $P<0.001$). Results from linear regression models using the continuous LE8 score as a supportive outcome were consistent, with a 1-point increase in PHQ-9 score associated with a β coefficient of −0.59 (95% CI: −0.67 to −0.52), and depression associated with a β coefficient of −6.33 (95% CI: −7.43 to −5.23; both $P<0.001$) (Table 2).

Higher DI-GM scores were significantly associated with better cardiovascular health. In analyses with LE8 category as the primary outcome, each 1-point increase in DI-GM score was associated with a 46% higher cumulative odds of being in a higher LE8 category (OR=1.46, 95% CI: 1.42–1.51, $P<0.001$). Compared with participants in the DI-GM score group of 0–3, those in the 4, 5, and ≥6 groups showed progressively higher odds of being in a higher LE8 category (ORs=1.81, 2.92, and 5.40, respectively), demonstrating a significant dose-response trend (P for trend<0.001). Findings from linear regression models using the continuous LE8 score as the supportive outcome were consistent (β=2.34 per 1-point increase in DI-GM score, 95% CI: 2.19–2.50, $P<0.001$) (Table 3). Further decomposition of the DI-GM into beneficial and unfavorable component scores revealed opposite directions of association with LE8. In addition, the joint exposure analysis of depression and DI-GM is

presented in Supplementary Table S2, with findings consistent with an independent and cumulative association pattern between the two factors. The global test of the proportional-odds assumption was rejected in all LE8 category models (all $P < 0.001$; Supplementary Table S7); partitioning the test showed that the departure was driven almost entirely by the covariates, particularly age, sex and race/ethnicity ($\chi^2 = 62.8–88.6$, 6 df, $P < 0.001$), whereas the assumption was not violated for the primary exposure (PHQ-9 score: $\chi^2 = 3.4$, 1 df, $P = 0.065$; depression ≥10: $P = 0.111$). For DI-GM the assumption was violated ($\chi^2 = 22.8$, 1 df, $P < 0.001$): the association was stronger for attaining high cardiovascular health (OR 1.52, 95% CI 1.47–1.57) than for avoiding low cardiovascular health (OR 1.29, 1.21–1.38), although the direction was identical and the dose–response gradient monotonic at both cut points.

Restricted cubic spline (RCS) analyses were further conducted to assess the dose–response relationships of depressive symptoms and DI-GM scores with LE8 levels. The results demonstrated an overall monotonic association for both exposures with the likelihood of achieving higher LE8 levels (both P-overall<0.001): as PHQ-9 scores increased, the probability of attaining higher LE8 levels continuously decreased, whereas this probability progressively increased with higher DI-GM scores (Figure 2). Although the test for nonlinearity reached statistical significance under the large sample size (Supplementary Table S3), the spline curves did not reveal distinct plateau phases, inflection points, or threshold effects with clinical interpretability, and the overall patterns remained approximately linear and monotonic. Therefore, PHQ-9 and DI-GM were further modeled as continuous variables in subsequent regression analyses to fully utilize their continuous distributions and capture the stability of these associations across the entire exposure range.

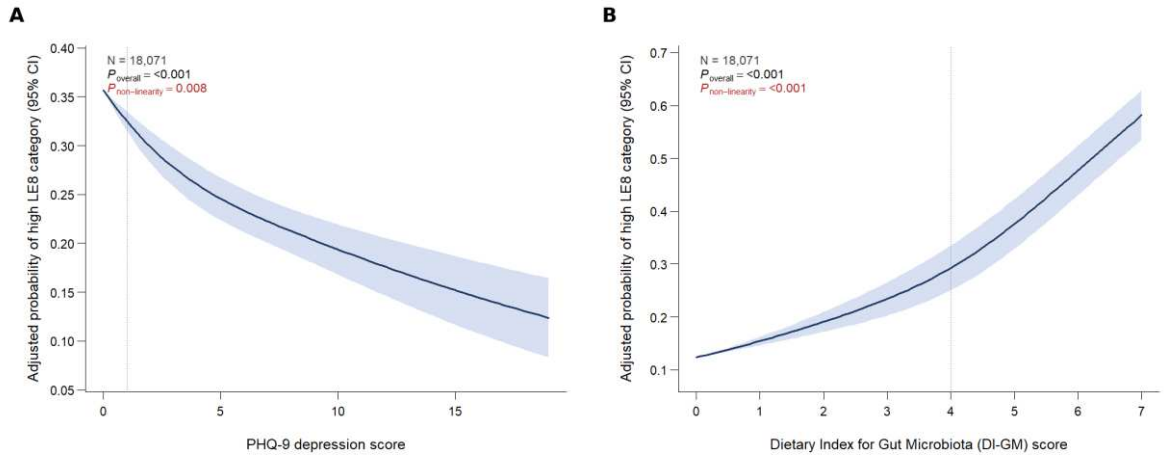


Figure 2. Restricted cubic spline (RCS) dose–response relationships between depression (PHQ-9 score; A) and the total Dietary Index for Gut Microbiota (DI-GM) score (B) and the probability of being in a higher Life's Essential 8 (LE8) cardiovascular health category.
The y-axis represents the adjusted probability of belonging to a higher LE8 category. Solid lines indicate point estimates, and shaded areas represent 95% confidence intervals (CIs). Vertical dashed lines denote the reference values (A: PHQ-9 = 1; B: DI-GM = 4). Panel A corresponds to the total effect pathway (depression → LE8), whereas Panel B corresponds to the mediator-to-outcome pathway (path *b*: DI-GM → LE8). Models were adjusted for age, sex, race/ethnicity, marital status, educational attainment, and the poverty-to-income ratio (PIR), while accounting for the complex NHANES sampling design (N = 18,071).

Table 2 Association between depression (PHQ-9) and LE8 cardiovascular health

Characteristics	Crude model		Adjusted model	
	Estimate (95% CI)	***P* value**	**Estimate (95% CI)**	***P* value**
Primary outcome: LE8 category (ordinal logistic regression), OR (95% CI)				
PHQ-9 score (per 1-point)	0.92 (0.90, 0.93)	<0.001	0.91 (0.90, 0.93)	<0.001
Depression: No	Ref		Ref	
Depression: Yes	0.36 (0.27, 0.48)	<0.001	0.39 (0.29, 0.51)	<0.001
Supporting outcome: LE8 score (survey-weighted linear regression), β (95% CI)				
PHQ-9 score (per 1-point)	−0.64 (−0.72, −0.56)	<0.001	−0.59 (−0.67, −0.52)	<0.001
Depression: No	Ref		Ref	
Depression: Yes	−7.32 (−8.56, −6.09)	<0.001	−6.33 (−7.43, −5.23)	<0.001

Abbreviations: CI, confidence interval; LE8, Life's Essential 8; OR, odds ratio; PHQ-9, Patient Health Questionnaire-9.
[a] The crude model was unadjusted for covariates. The adjusted model included age, sex, race/ethnicity, marital status, educational attainment, and the poverty-to-income ratio (PIR).
[b] Depression was defined as a PHQ-9 score ≥10, with participants without depression serving as the reference group. A β coefficient <0 and an OR <1 both indicate poorer cardiovascular health.
[c] All analyses accounted for the complex NHANES sampling design and corresponding survey weights.

Table 3 Association between DI-GM and LE8 cardiovascular health

Characteristics	Crude model		Adjusted model	
	Estimate (95% CI)	***P* value**	**Estimate (95% CI)**	***P* value**
Primary outcome: LE8 category (ordinal logistic regression), OR (95% CI)				
DI-GM score (per 1-point)	1.42 (1.38, 1.46)	<0.001	1.46 (1.42, 1.51)	<0.001
DI-GM 0–3	Ref		Ref	
DI-GM 4	1.71 (1.49, 1.97)	<0.001	1.81 (1.58, 2.08)	<0.001
DI-GM 5	2.64 (2.31, 3.02)	<0.001	2.92 (2.54, 3.36)	<0.001
DI-GM ≥6	4.62 (4.07, 5.25)	<0.001	5.40 (4.67, 6.23)	<0.001
Trend test		<0.001		<0.001
DI-GM beneficial score (per 1-point increase)	1.55 (1.50, 1.61)	<0.001	1.67 (1.60, 1.74)	<0.001
DI-GM unfavorable score (per 1-point increase)	0.72 (0.68, 0.75)	<0.001	0.71 (0.67, 0.75)	<0.001
Supporting outcome: LE8 score (survey-weighted linear regression), β (95% CI)				
DI-GM score (per 1-point)	2.39 (2.22, 2.55)	<0.001	2.34 (2.19, 2.50)	<0.001
DI-GM 0–3	Ref		Ref	
DI-GM 4	3.66 (2.86, 4.47)	<0.001	3.69 (2.98, 4.40)	<0.001
DI-GM 5	6.92 (6.13, 7.71)	<0.001	6.85 (6.13, 7.57)	<0.001
DI-GM ≥6	10.53 (9.80, 11.26)	<0.001	10.19 (9.49, 10.89)	<0.001
Trend test		<0.001		<0.001
DI-GM beneficial score (per 1-point increase)	3.04 (2.82, 3.27)	<0.001	3.14 (2.92, 3.36)	<0.001
DI-GM unfavorable score (per 1-point increase)	-2.38 (-2.68, -2.08)	<0.001	-2.24 (-2.52, -1.96)	<0.001

Abbreviations: CI, confidence interval; DI-GM, Dietary Index for Gut Microbiota; LE8, Life's Essential 8; OR, odds ratio.
[a] The crude model was unadjusted for covariates. The adjusted model included age, sex, race/ethnicity, marital status, educational attainment, and the poverty-to-income ratio (PIR).
[b] The DI-GM score ranged from 0 to 13 and was categorized into four groups (0–3, 4, 5, and ≥6), with 0–3 as the reference group. Associations of the beneficial and unfavorable component scores were also examined. *P* for trend was obtained from a design-based Wald test (1 df, df = 94) on a continuous variable coded as the median DI-GM value within each category (2, 4, 5 and 6).

[c] All analyses accounted for the complex NHANES sampling design and incorporated the corresponding survey weights.

3. Mediation of the depression–LE8 association by DI-GM

Mediation analysis within the counterfactual framework indicated that DI-GM significantly mediated the association between depression and cardiovascular health status measured by LE8 (Figure 3, Table 4). In the mediation model, higher levels of depressive symptoms were associated with lower DI-GM scores (path *a*: each 1-point increase in PHQ-9 score was associated with a 0.013-point decrease in DI-GM, 95% CI −0.022 to −0.003, *P*=0.007), whereas higher DI-GM scores were associated with better LE8 profiles (path *b*: OR=1.478, 95% CI 1.430–1.538, *P*<0.001).

In the overall population, the total effect of depression on LE8 cardiovascular health indicated that each 1-point increase in PHQ-9 score was associated with a lower likelihood of being in a higher LE8 category (TE: marginal cumulative OR=0.924, 95% CI 0.909–0.939, *P*<0.001), of which the pure natural direct effect was OR=0.928 (0.913–0.943, *P*<0.001). The natural indirect effect mediated through DI-GM was statistically significant (TNIE: OR=0.9958, 95% CI 0.9928–0.9990, *P*=0.008), accounting for 5.08% (95% CI 1.35–8.87%) of the total association. On the probability scale the same decomposition gave a total effect of −1.73 (−2.10 to −1.37) percentage points in the probability of attaining high cardiovascular health, of which −0.09 (−0.16 to −0.02) percentage points were mediated by DI-GM. The exposure-by-mediator interaction was not statistically significant (β=−0.0035, 95% CI −0.0144 to 0.0069, *P*=0.52), so the pure and total natural indirect effects were nearly identical and the controlled direct effect (−1.69 percentage points) closely approximated the natural direct effect.

The mediation effect remained robust under different specifications of both the exposure and outcome variables. Across four analytical combinations using either continuous PHQ-9 scores or depression status as the exposure, and LE8 categories or continuous LE8 scores as the outcome, the direction of the indirect effect remained consistent and statistically significant, with the proportion mediated ranging from 3.66% to 5.38% (Table 4; Supplementary Table S4).

It should be noted that the dietary component of LE8 partially overlaps with DI-GM, which may have inflated the association between DI-GM and LE8 to some extent (i.e., pathway *b*). Sensitivity analyses showed that when LE8 was re-estimated after excluding the dietary

component (7-item LE8), the effect estimate for pathway *b* was attenuated from OR=1.478 to OR=1.191, and the mediation proportion decreased from 5.08% to 2.65%. However, the natural indirect effect remained statistically significant (*P*=0.012), suggesting that the observed mediation was not entirely attributable to the overlap in dietary components (Supplementary Table S5).

Table 4 Counterfactual decomposition of the association between depression and LE8 cardiovascular health, with DI-GM as the mediator

Specification (exposure → outcome)	Effect scale	Total effect (TE), (95% CI)	Natural direct effect (PNDE), (95% CI)	Natural indirect effect (TNIE), (95% CI)	*P* value	% mediated (95% CI)
PHQ-9 per 1 point → LE8 category (primary)	Marginal cumulative OR	0.924 (0.909, 0.939)	0.928 (0.913, 0.943)	0.9958 (0.9928, 0.9990)	0.008	5.08 (1.35, 8.87)
PHQ-9 per 1 point → LE8 score	β (points)	−0.598 (−0.673, −0.527)	−0.568 (−0.638, −0.501)	−0.030 (−0.052, −0.008)	0.009	5.04 (1.34, 8.55)
Depression ≥10 → LE8 category (primary)	Marginal cumulative OR	0.414 (0.318, 0.529)	0.436 (0.334, 0.561)	0.9509 (0.9143, 0.9845)	0.013	3.66 (0.94, 7.74)
Depression ≥10 → LE8 score	β (points)	−6.355 (−7.520, −5.260)	−6.013 (−7.114, −4.977)	−0.342 (−0.642, −0.104)	0.012	5.38 (1.57, 9.67)

Abbreviations: CI, confidence interval; DI-GM, Dietary Index for Gut Microbiota; LE8, Life's Essential 8; OR, odds ratio; PHQ-9, Patient Health Questionnaire-9; PNDE, pure natural direct effect; TE, total effect; TNIE, total natural indirect effect.
[a] Effects were identified by g-computation within the counterfactual framework, with the mediator distribution represented by the weighted empirical residual distribution and an exposure-by-mediator interaction included in the outcome model. For ordinal outcomes, effects are marginal cumulative odds ratios and the decomposition is exact (OR_TE = OR_PNDE × OR_TNIE); for continuous outcomes, effects are on the natural (β) scale and TE = PNDE + TNIE.
[b] 95% CIs are percentile intervals from 500 Rao–Wu rescaled bootstrap replicates preserving the NHANES strata and primary sampling units; P values are design-based (df = 94).
[c] Effects for the ordinal outcome are marginal (standardised over the covariate distribution) and therefore differ numerically from the conditional odds ratios in Table 2, as expected given the non-collapsibility of the odds ratio. All models adjust for age, sex, race/ethnicity, marital status, education and PIR.

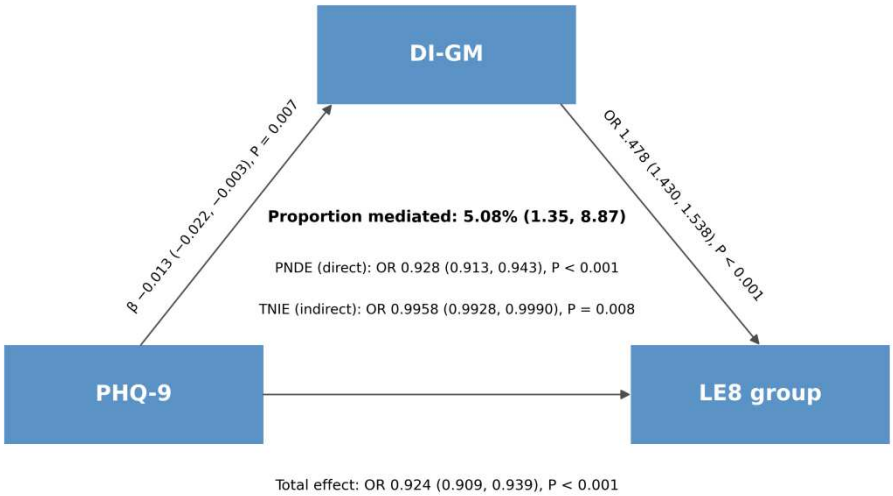


Figure 3. Counterfactual mediation pathway of DI-GM in the association between depression and LE8 cardiovascular health (overall population; primary analysis specification: continuous PHQ-9 score → DI-GM → LE8 category).
Path *a* represents the association between the exposure and the mediator (PHQ-9 → DI-GM; β=−0.013, 95% CI −0.022 to −0.003, *P*=0.007), and path *b* the association between the mediator and the outcome (DI-GM → LE8, from the outcome model including the exposure-by-mediator interaction; OR=1.478, 95% CI 1.430–1.538, *P*<0.001). Effects were identified by g-computation within the counterfactual framework and are expressed as marginal cumulative odds ratios: total effect 0.924 (0.909, 0.939); pure natural direct effect 0.928 (0.913, 0.943); total natural indirect effect 0.9958 (0.9928, 0.9990), P=0.008;

proportion mediated 5.08% (1.35%, 8.87%). Because the decomposition is multiplicative on this scale, the total effect equals the product of the direct and indirect odds ratios. 95% CIs are percentile intervals from 500 Rao–Wu rescaled bootstrap replicates. The model was adjusted for age, sex, race/ethnicity, marital status, educational attainment, and poverty-to-income ratio (PIR), and accounted for the complex sampling design of NHANES (N=18,071).

To identify populations in which this mediation pathway was more pronounced, prespecified effect modification analyses were conducted (Figure 4). Among women, the mediating effect of DI-GM was statistically significant (TNIE OR=0.9946, 95% CI 0.9912–0.9982; proportion mediated 6.0%, *P*=0.004), whereas no significant mediation effect was observed among men (proportion mediated 2.9%, *P*=0.450). This difference was primarily attributable to path *a*: the association between depression and lower DI-GM scores was significant among women (β=−0.019, *P*=0.003) but not among men (*P*=0.446).

Similarly, the mediation effect was stronger among participants with gastrointestinal disease (TNIE OR=0.9847, 95% CI 0.9760–0.9930; proportion mediated 14.2%, *P*<0.001), whereas it did not reach statistical significance among those without gastrointestinal disease (proportion mediated 3.4%, *P*=0.105). This difference was likewise driven by a stronger path a association (the association between depression and DI-GM among individuals with gastrointestinal disease: β=−0.053, *P*<0.001). Further exploratory analysis suggested that the proportion mediated was highest among participants who were both women and had gastrointestinal disease (approximately 23.8%, *P*=0.004). Given the limited sample size of this subgroup (n=720), these findings should be interpreted cautiously as hypothesis-generating evidence.

Additionally, the indirect effect of DI-GM was statistically significant among participants aged 40–59 years (proportion mediated 10.0%, *P*=0.009) and among those with a poverty-to-income ratio (PIR) >3.5 (15.0%, *P*=0.004). However, when PHQ-9 was replaced with a binary variable (PHQ-9 ≥10) in the modeling analysis, these effects were attenuated and no longer reached statistical significance. Stratified analyses based on major comorbidities showed that the indirect effect of DI-GM was not statistically significant among participants with diabetes (*P*=0.444), kidney disease (*P*=0.592), sleep problems (*P*=0.062), stroke (*P*=0.431), or cancer (*P*=0.156), whereas it remained significant in the corresponding subgroups without these conditions. Across standard demographic subgroups, including age, race/ethnicity, marital status, educational attainment, and PIR, the direction of the indirect effects was generally consistent, with most subgroup estimates below the null value of 1 (full results are presented in Supplementary Table S6 and Supplementary Figure S1). Importantly, differences in statistical significance across subgroups do not necessarily indicate differences

in the magnitude of effects between subgroups. Because formal interaction tests between subgroups were not performed, these patterns should be interpreted as exploratory. Overall, although the DI-GM-mediated pathway accounted for a relatively small proportion of the association in the overall population, it appeared to be more prominent among women and individuals with gastrointestinal disease.

Subgroup	Indirect OR (95% CI)	P value
Full sample		
Overall	0.9958 (0.9930, 0.9990)	0.007
Age group		
20-39	0.9982 (0.9945, 1.0018)	0.352
40-59	0.9909 (0.9843, 0.9970)	0.009
>=60	0.9885 (0.9747, 1.0007)	0.089
Sex		
Male	0.9980 (0.9926, 1.0033)	0.450
Female	0.9946 (0.9912, 0.9982)	0.004
Race/ethnicity		
Mexican American	1.0067 (1.0001, 1.0142)	0.072
Other Hispanic	0.9938 (0.9869, 1.0013)	0.100
Non-Hispanic White	0.9954 (0.9912, 0.9998)	0.033
Non-Hispanic Black	0.9954 (0.9900, 1.0002)	0.076
Other Race	0.9944 (0.9869, 1.0009)	0.139
Marital status		
Unmarried	0.9979 (0.9940, 1.0008)	0.274
Married/partnered	0.9938 (0.9896, 0.9980)	0.006
Education		
Less than high school	0.9965 (0.9908, 1.0007)	0.191
High school	1.0025 (0.9975, 1.0070)	0.300
Above high school	0.9921 (0.9881, 0.9963)	<0.001
PIR category		
<=1.3	0.9995 (0.9972, 1.0020)	0.738
1.3-3.5	0.9975 (0.9928, 1.0019)	0.287
>3.5	0.9879 (0.9809, 0.9953)	0.004
Diabetes status		
No diabetes	0.9963 (0.9928, 0.9998)	0.030
Borderline	0.9952 (0.9821, 1.0088)	0.474
Diabetes	0.9965 (0.9858, 1.0037)	0.444
GI disease		
No	0.9974 (0.9947, 1.0006)	0.105
Yes	0.9847 (0.9760, 0.9930)	<0.001
Kidney disease		
No	0.9957 (0.9929, 0.9989)	0.006
Yes	1.0044 (0.9866, 1.0211)	0.592
Sleep problem		
No	0.9969 (0.9916, 1.0011)	0.191
Yes	0.9959 (0.9917, 1.0001)	0.062
Stroke		
No	0.9961 (0.9932, 0.9993)	0.014
Yes	0.9934 (0.9755, 1.0058)	0.431
Cancer		
No	0.9962 (0.9928, 0.9993)	0.018
Yes	0.9926 (0.9826, 1.0036)	0.156
Exploratory: Sex x GI disease		
Male | No	0.9991 (0.9923, 1.0047)	0.768
Male | Yes	0.9924 (0.9774, 1.0015)	0.262
Female | No	0.9964 (0.9931, 1.0001)	0.056
Female | Yes	0.9761 (0.9602, 0.9900)	0.004

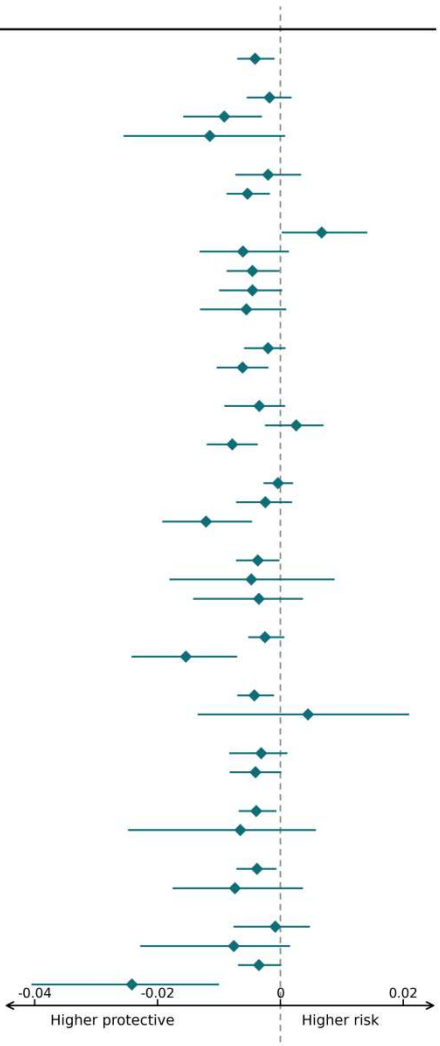


Figure 4. Subgroup forest plot of the natural indirect effects of DI-GM mediating the association between depression and LE8 cardiovascular health.
Diamonds represent the estimated natural indirect effects (marginal cumulative odds ratios) for each subgroup, and horizontal lines indicate 95% confidence intervals (CIs); the horizontal axis is on the log-odds-ratio scale. The primary mediation specification was continuous PHQ-9 score → DI-GM → LE8 category. Each subgroup was analysed separately by domain estimation within the counterfactual framework using g-computation; models were adjusted for age, sex, race/ethnicity, marital status, educational attainment, and PIR (excluding the stratifying variable), while accounting for the complex sampling design of NHANES. 95% CIs are percentile intervals from 200 Rao–Wu rescaled bootstrap replicates.

Discussion

This study used nationally representative data from U.S. adults participating in NHANES 2007–2018 to systematically investigate the relationships among depression, the Dietary Index for Gut Microbiota (DI-GM), and LE8 cardiovascular health from a mediation perspective. Three major findings emerged. First, a greater burden of depressive symptoms was significantly associated with poorer LE8 cardiovascular health, whereas higher DI-GM scores were associated with better cardiovascular health; these associations were directionally opposite and remained independently significant. Second, DI-GM significantly mediated the association between depression and LE8, although the proportion mediated was relatively modest (approximately 5.1%). Third, this mediation pathway was more pronounced among women and individuals with gastrointestinal disease. Collectively, these findings provide population-level epidemiological evidence supporting the potential involvement of the "brain–gut–heart" axis.

The association between depression and poorer cardiovascular health is consistent with previous evidence. Depression has been associated not only with individual cardiovascular risk factors but may also contribute to impaired overall cardiovascular health through the accumulation and progression of multiple risk factors[6,7]. The American Heart Association has also emphasized the bidirectional relationship between psychological health and cardiovascular health[8]. By considering depression as the exposure and LE8, which incorporates both health behaviors and physiological factors, as the outcome, this study further supports this comprehensive association. However, given the cross-sectional design, the observed associations may reflect an adverse effect of depression on cardiovascular health, while the possibility that poorer cardiovascular health may contribute to worsening depressive symptoms cannot be excluded.

Higher DI-GM scores were associated with better LE8 cardiovascular health, and the beneficial and unfavorable components showed opposite directions of association, strengthening the internal consistency of the findings. These results are consistent with recent NHANES-based evidence[23]. Mechanistically, dietary patterns rich in dietary fiber, whole grains, and fermented foods may contribute to metabolic and immune homeostasis by promoting short-chain fatty acid production and maintaining intestinal barrier integrity[13,20]. In contrast, unfavorable components such as red meat and processed meat may be metabolized by the gut microbiota to produce trimethylamine *N*-oxide (TMAO), which has been associated with atherosclerosis and increased cardiovascular risk[19]. It should be emphasized that DI-GM is a gut microbiota–related dietary index constructed based on

dietary intake rather than a direct measure of gut microbiota composition or function. Therefore, the present findings more accurately reflect the association between a gut microbiota–favorable dietary pattern and better cardiovascular health, rather than alterations in the gut microbiota itself. In addition, the DI-GM partially overlaps with the dietary component of LE8, which may contribute to an overestimation of the association between DI-GM and LE8 (i.e., path *b*). Therefore, these findings should be interpreted with caution.

Mediation analysis suggested that DI-GM partially mediated the association between depression and LE8 cardiovascular health. This finding is consistent with the hypothesis that depression may be accompanied by a less favorable gut microbiota–related dietary pattern, which may subsequently be associated with poorer cardiovascular health. The relationship between depression and gut microbiota–related dietary patterns is biologically plausible: individuals with depression often exhibit poorer diet quality and alterations in gut microbiota diversity[14,15]. Moreover, depression and inflammation may mutually reinforce each other[27], potentially affecting systemic metabolism and cardiovascular health through the "microbiota–gut–brain" axis. However, the relatively modest proportion mediated (approximately 5.1%) suggests that the majority of the association between depression and cardiovascular health may operate through pathways beyond gut microbiota–related dietary patterns, such as inflammation, neuroendocrine dysregulation, behavioral factors, and other metabolic pathways. Notably, although the mediated proportion was limited, DI-GM represents a measurable pathway that is, in principle, modifiable through dietary interventions, thereby providing unique potential value from a public health perspective. Because the effects were estimated within the counterfactual framework, the decomposition into direct and indirect components is exact on both the risk-difference and odds-ratio scales, and the proportion mediated does not rely on the approximate additivity assumed by the traditional product-of-coefficients approach.

A further observation concerns the shape of the DI-GM–LE8 association across the cardiovascular health spectrum. The association was stronger for attaining high cardiovascular health than for avoiding low cardiovascular health (Supplementary Table S7), which is of substantive interest: a gut-microbiota-favourable diet may contribute more to reaching optimal cardiovascular health than to preventing the poorest health states, which are more likely to be dominated by established clinical risk factors. The pooled cumulative odds

ratio should therefore be read as a weighted average of the cut-point-specific associations rather than as a single effect applying uniformly across the spectrum.

Effect modification analyses showed that the mediation pathway was more pronounced among women and individuals with gastrointestinal disease, and this difference was primarily driven by path *a* (the association between depression and lower DI-GM scores). This finding is biologically plausible. On the one hand, the association between depression and cardiovascular risk among women may be influenced by multiple factors, including hormonal, inflammatory, and behavioral pathways[28]. On the other hand, gastrointestinal diseases are often accompanied by dietary alterations, impaired intestinal barrier function, and gut microbiota dysbiosis, which may increase the sensitivity of the diet–microbiota pathway in this population[29]. Although the highest proportion mediated was observed among individuals who were both women and had gastrointestinal disease (approximately 23.1%), this subgroup had a limited sample size (n=720), and formal statistical comparisons of mediated effects across subgroups were not performed. Therefore, this finding should be interpreted as a biologically plausible, hypothesis-generating result that requires validation in independent populations and prospective studies. In contrast, subgroup analyses stratified by age, economic status, and comorbid conditions did not provide clear evidence of effect modification. Although the indirect effect of DI-GM was statistically significant among participants aged 40–59 years and those with a poverty-to-income ratio (PIR) >3.5 in the primary model specification, these associations were attenuated and lost statistical significance when depression was modeled as a binary variable. Moreover, the indirect effect of DI-GM did not reach statistical significance in any of the comorbidity subgroups (diabetes, kidney disease, sleep problems, stroke, and cancer). Therefore, these stratified findings should be considered exploratory, and no stable differential mediating effect of DI-GM across subgroups can be established.

The findings of this study have potential public health implications. Both PHQ-9 and LE8 are relatively simple assessment tools. Integrating depressive symptoms, overall cardiovascular health, and gut microbiota–related dietary patterns may help identify populations with concurrent psychological and cardiovascular risk factors. Moreover, the dietary exposure reflected by DI-GM is potentially modifiable, and improving dietary patterns among populations in which this pathway is more prominent warrants further evaluation in prospective and intervention studies. However, given the cross-sectional nature of the current

evidence, it cannot be concluded that improving DI-GM would necessarily lead to better cardiovascular health, nor is the evidence sufficient to support specific clinical dietary recommendations at present.

This study has several strengths. First, it utilized a large, nationally representative sample from NHANES and accounted for its complex sampling design. Second, the use of both LE8 categories and continuous LE8 scores as primary and supportive outcomes, respectively, enhanced the consistency of the findings. Third, by integrating association analyses, dose–response analyses, mediation analyses, and prespecified subgroup analyses, this study provided a comprehensive characterization of the relationships among depression, gut microbiota–related dietary patterns, and overall cardiovascular health.

Several limitations should also be acknowledged. First, the cross-sectional design precludes the determination of temporal relationships, prevents causal inference, and allows the possibility of reverse associations. Second, DI-GM was calculated based on self-reported dietary recall data and may therefore be subject to recall bias and measurement error. Moreover, DI-GM represents only a proxy measure of a gut microbiota–related dietary pattern and cannot substitute for direct assessments of gut microbiota composition or metabolite profiles. Third, the PHQ-9 is a screening instrument for depressive symptoms and does not fully replace structured clinical diagnostic assessments. Fourth, although the models adjusted for multiple sociodemographic factors, residual confounding from factors such as total energy intake, medication use, and chronic inflammation cannot be excluded. Fifth, a substantial number of participants were excluded due to missing DI-GM or other variables, and the complete-case analysis may have introduced selection bias. Sixth, the partial overlap between DI-GM and the dietary component of LE8 may have strengthened their association and potentially led to overestimation of the mediation effect. Seventh, the causal interpretation of the natural direct and indirect effects rests on four identification assumptions: no unmeasured confounding of the exposure–outcome, mediator–outcome or exposure–mediator relationships, and no mediator–outcome confounder affected by the exposure; because NHANES is cross-sectional, the temporal ordering from depressive symptoms to diet to cardiovascular health cannot be verified, so the estimates should be read as the mediated proportion that would hold if the assumed ordering were correct rather than as established causal effects. Eighth, the proportional-odds assumption was formally rejected; given the sample size such tests are highly sensitive to minor departures, the assumption held

for the primary exposure, the cut-point-specific associations were consistent in direction throughout, and the linear models for the continuous LE8 score, which do not rely on this assumption, yielded the same conclusions, including an almost identical mediated proportion (5.04% versus 5.08%); the pooled cumulative odds ratios should nonetheless be interpreted as summary measures across the cardiovascular health spectrum. Finally, subgroup analyses involved multiple comparisons, and the findings among women with gastrointestinal disease require further external validation.

Conclusion

This study used nationally representative data from U.S. adults participating in NHANES 2007–2018 to systematically evaluate the relationships among depression, gut microbiota–related dietary patterns (DI-GM), and LE8 cardiovascular health from a mediation perspective. The findings showed that a greater burden of depressive symptoms was associated with poorer LE8 cardiovascular health, whereas higher DI-GM scores were associated with better cardiovascular health. DI-GM partially mediated the association between depression and cardiovascular health; although the proportion mediated was relatively modest, this pathway was more pronounced among women and individuals with gastrointestinal disease. These findings provide indirect epidemiological evidence for understanding cardiovascular health from an integrated "brain–gut–heart" perspective and suggest that gut microbiota–related dietary patterns may represent a potential mediating pathway through which depression influences cardiovascular health. Given the cross-sectional design of this study, the temporal sequence and underlying mechanisms of these associations require further validation through longitudinal cohort studies and dietary intervention trials incorporating gut microbiota sequencing, microbial metabolite profiling, and inflammatory biomarker measurements.

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Author contributions statement

H.Z. developed the methodology and supervised the work. J.S. performed analysis and wrote the original draft. Z.Z., Z.J. and L.J. curated the data, M.J. and S.F. reviewed and edited the manuscript. All authors reviewed and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics and Consent to Participate declarations: The NHANES protocols were approved by the NCHS Research Ethics Review Board, and written informed consent was obtained from all participants.

Data availability statement

The datasets analyzed in this study are publicly available from the National Health and Nutrition Examination Survey (NHANES), conducted by the National Center for Health Statistics (NCHS), Centers for Disease Control and Prevention (CDC). The six cycles used in this analysis can be accessed directly at the following links: NHANES 2007–2008 (https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/default.aspx?BeginYear=2007), 2009–2010 (https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/default.aspx?BeginYear=2009), 2011–2012 (https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/default.aspx?BeginYear=2011), 2013–2014 (https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/default.aspx?BeginYear=2013), 2015–2016 (https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/default.aspx?BeginYear=2015), and

2017–2018

(https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/default.aspx?BeginYear=2017); an index of all cycles together with their documentation and codebooks is available at https://wwwn.cdc.gov/nchs/nhanes/Default.aspx. No new data were generated or analyzed in this study.

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