Aryaeian *et al. Nutrition Journal* (2025) 24:165
https://doi.org/10.1186/s12937-025-01227-0

Nutrition Journal

REVIEW Open Access

Effect of egg consumption on circulating choline, betaine, and trimethylamine n-oxide in adults: a systematic review and meta-analysis of randomized controlled trials

Naheed Aryaeian[1], Azadeh Heydarian[1], Negin Tahvilian[2,3], Hossein Hosseini[4], Amirhossein Ramezani Ahmadi[5] and Seyedeh Hajar Davarpanah[1*]

Abstract

Background and objective Eggs are rich in choline and other methyl donors that may influence metabolic health, yet their effects on circulating metabolites such as choline, betaine, and trimethylamine N-oxide (TMAO) remain unclear. Understanding these effects is critical due to potential links with cardiometabolic risk. This study aimed to systematically evaluate and quantitatively synthesize the effects of egg consumption on plasma levels of choline, betaine, and TMAO in randomized controlled trials (RCTs), and to explore potential effect modifiers.

Methods A systematic search of electronic databases was conducted to identify RCTs examining the effects of egg consumption on circulating choline, betaine, and TMAO. Data were pooled using random-effects meta-analyses. Subgroup analyses were performed based on age, body mass index, health status, study design, duration, and egg dosage. Meta-regression was conducted to assess the influence of age, dose, and duration. Publication bias was evaluated using Egger's and Begg's tests, funnel plots, and trim-and-fill analysis. The certainty of evidence was assessed using the NutriGRADE tool, which is specifically recommended for nutrition meta-analyses.

Results Six RCTs ($n=5$ for betaine and TMAO; $n=6$ for choline) were included. Multi-arm trials were combined into single pairwise comparisons per outcome, as per Cochrane Handbook Sect. 23.3.4, to avoid unit-of-analysis errors. No significant overall effects were observed for choline (WMD = 0.10; 95% CI: −0.92 to 1.12; $p=0.847$), betaine (WMD = 0.21; 95% CI: −8.50 to 8.92; $p=0.962$), or TMAO (WMD = −0.08; 95% CI: −0.47 to 0.32; $p=0.692$). High heterogeneity was noted for choline and betaine ($I^2 > 85\%$), but low for TMAO ($I^2 = 23\%$). Subgroup analyses showed significant choline increases among older adults (>45 years) and individuals with metabolic syndrome or obesity. Meta-regression revealed a negative association between egg dose and both choline and betaine levels ($p<0.001$). Publication bias was suggested for betaine but not for choline or TMAO. The NutriGRADE assessment indicated moderate certainty of evidence for choline (score 7.75), betaine (7.05), and TMAO (7.25), with downgrades mainly due to imprecision and heterogeneity.

*Correspondence:
Seyedeh Hajar Davarpanah
Davarpanah.nut65@gmail.com

Full list of author information is available at the end of the article

© The Author(s) 2025. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

Conclusions No clear effect of egg consumption on circulating choline, betaine, or TMAO levels could be demonstrated. Age and metabolic status may influence choline responses, and higher egg doses may attenuate choline and betaine levels. Based on NutriGRADE, the overall certainty of evidence was rated as moderate for all three outcomes. Further long-term, high-quality RCTs are needed to clarify these associations.

Keywords Eggs, Choline, Betaine, TMAO, Meta-analysis, Randomized controlled trials, Cardiometabolic health, Methyl donors

Introduction

Eggs are a widely consumed, nutrient-dense food, recognized for their high biological value protein, fat-soluble vitamins, minerals, and particularly, choline—an essential nutrient involved in membrane structure, neurotransmitter synthesis, and one-carbon metabolism [1, 2]. In recent years, eggs have become the focus of nutritional debate due to their dual profile: on one hand, they provide important bioactive nutrients; on the other, they are rich in cholesterol and choline compounds that may contribute to the generation of potentially atherogenic metabolites [3, 4].

One such metabolite is trimethylamine N-oxide (TMAO), which has emerged as a novel biomarker in cardiovascular research. TMAO is produced in the liver via flavin-containing monooxygenase 3 (FMO3)-mediated oxidation of trimethylamine (TMA), which itself is generated by gut microbial metabolism of dietary precursors such as choline, betaine (also known as trimethylglycine), and L-carnitine [5–7]. The interest in TMAO stems from accumulating evidence suggesting its possible involvement in atherosclerosis, endothelial dysfunction, platelet hyperreactivity, and cardiovascular events. However, the causal role of TMAO in human health is still under investigation and remains a topic of ongoing scientific controversy [8, 9].

Given that eggs are one of the richest dietary sources of choline, especially in the form of phosphatidylcholine (lecithin), their potential to elevate circulating TMAO levels has raised concerns about their cardiometabolic safety [10, 11]. Several randomized controlled trials (RCTs) have investigated the effect of egg consumption on TMAO and related metabolites, yielding conflicting results. Some studies report that egg intake modestly increases TMAO levels, while others suggest no significant effect, possibly due to variations in gut microbiota composition, genetic differences in FMO3 activity, baseline choline status, or differences in study design including duration, dose, and background diet [12–14].

Moreover, choline and its metabolites such as betaine are not merely precursors to TMAO; they also play critical physiological roles. Choline is involved in homocysteine remethylation through its oxidation product betaine, which acts as a methyl donor. Adequate intake of choline and betaine has been associated with favorable outcomes such as reduced homocysteine concentrations, improved hepatic function, and cognitive health. Thus, the interpretation of their effects should consider both potential risks (i.e., TMAO formation) and benefits [15–17].

Despite the growing number of RCTs assessing the metabolic impact of egg consumption, no systematic review has yet synthesized findings specifically on TMAO and its related metabolites. Clarifying how eggs influence circulating levels of TMAO, choline, and betaine is important for informing dietary recommendations, particularly in populations at increased cardiometabolic risk. Therefore, the present systematic review and meta-analysis aimed to evaluate the effects of egg consumption on these metabolites in adults.

Methods

Protocol and registration

This systematic review and meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines (Supplementary Table 1). The review protocol was prospectively registered in the International Prospective Register of Systematic Reviews (PROSPERO; registration ID: CRD420251047728).

Eligibility criteria

This systematic review and meta-analysis followed the PICO (Population, Intervention, Comparator, Outcome) framework to define study eligibility. Studies were included if they met the following criteria: [1] Design: Randomized controlled trials (RCTs), including both parallel and crossover designs [2]. Population: Adult participants aged ≥ 18 years, regardless of health status or baseline metabolic condition [3]. Intervention: Consumption of eggs in any form, including whole eggs, egg yolk, or egg white [4]. Comparator: Any control group consuming a non-egg-based intervention or standard diet [5]. Outcomes: At least one of the following biomarkers was reported, plasma or serum concentrations of TMAO, choline, or betaine [6]. Language and Publication: Studies published in peer-reviewed journals in English with full-text availability.

Studies were excluded if they were not randomized trials, including cross-sectional studies, cohort studies, case reports, or quasi-experimental studies. In addition, animal studies, in vitro investigations, or studies conducted in children or pregnant women were excluded.

Trials with an intervention period of less than 24 h were excluded due to insufficient time to evaluate biomarker response. Further exclusion criteria included studies lacking extractable data (e.g., without means or standard deviations), conference abstracts, duplicate or overlapping datasets, and studies without access to full-text articles. In cases of multiple publications from the same trial, the most comprehensive or recent version was included.

Information sources and search strategy

A comprehensive and systematic literature search was conducted across three major scientific databases: PubMed (MEDLINE), Web of Science (WOS), and Scopus. These databases were selected due to their broad indexing of biomedical, nutritional, and clinical trials literature. The search included all available records from inception until April 2025, without restrictions on publication year.

The search strategy was developed using a combination of controlled vocabulary (e.g., MeSH terms in PubMed) and free-text terms, in accordance with the Peer Review of Electronic Search Strategies (PRESS) guidelines. Boolean operators (AND, OR) and truncation symbols were applied to optimize the sensitivity and specificity of the search.

The detailed search string used in all databases was as follows:

("egg consumption" OR "egg intake" OR "eggs" OR "whole egg" OR "dietary egg" OR "hen egg" OR "egg-based diet" OR "egg-rich diet" OR "egg-derived" OR "egg intervention" OR "egg product") AND ("Trimethylamine N-oxide" OR "TMAO" OR "trimethylamine oxide" OR "Choline" OR "phosphatidylcholine" OR "glycerophosphocholine" OR "free choline" OR "choline chloride" OR "choline bitartrate" OR "choline metabolism" OR "Betaine" OR "trimethylglycine" OR "betaine anhydrous" OR "dimethylglycine" OR "DMG") AND ("randomized controlled trial" OR "randomised controlled trial" OR "RCT" OR "randomized trial" OR "randomised trial" OR "intervention study" OR "controlled clinical trial" OR "clinical trial").

Study selection

Search results were imported into EndNote X20 for reference management and deduplication. Two independent reviewers (AH and NT) screened the titles and abstracts of all identified articles. Full-texts of potentially eligible studies were retrieved and assessed against inclusion criteria. Discrepancies were resolved through discussion or consultation with a third reviewer. In addition to database searches, manual searches of reference lists from included articles and relevant systematic reviews were conducted to identify additional eligible studies.

Data extraction

Data were independently extracted by two reviewers using a standardized and pre-designed data extraction form to ensure consistency and minimize bias. The extracted information included the following: study characteristics (first author, year of publication, country, study design, and sample size); participant characteristics (mean age, sex distribution, and baseline health status); details of the intervention (type of egg product used—whole egg, yolk, or white—along with dosage, frequency, and duration of consumption); and comparator characteristics. Outcome measures were collected as mean and standard deviation for plasma or serum levels of TMAO, choline, and betaine. Additional data on funding sources and potential conflicts of interest were also documented. For crossover trials, only outcome data from the end of each intervention period were extracted. When available, statistical adjustments were applied to account for within-subject correlations as reported by the original studies. In multi-arm trials (one two-arm [18] and one four-arm study [14]), relevant intervention and control arms were combined to create single pairwise comparisons per outcome, following Cochrane Handbook Sect. 23.3.4. For the four-arm study by Wilcox [14], arms A and B, which shared a common intervention but had different controls, were combined as one comparison, while arms C and D, which shared a common control but had different interventions, were combined as a separate comparison. This approach prevents double-counting and minimizes correlation between comparisons from the same study.

Risk of bias assessment

The risk of bias in included studies was assessed independently by two reviewers using the Cochrane Risk of Bias 2.0 tool (RoB 2) for randomized trials. Bias was assessed in five domains: randomization process, deviations from intended interventions, missing outcome data, measurement of outcomes, and selection of reported results.

Data synthesis and statistical analysis

Meta-analyses were performed using RevMan 5.4 and Stata 17.0 software. Specifically, Stata 17.0 was used for the main meta-analyses, meta-regression, and generation of all forest plots and funnel plots, while RevMan 5.4 was applied for cross-checking pooled effect estimates, sensitivity analyses, and creating risk-of-bias summary figures. For continuous outcomes, weighted mean differences (WMDs) and 95% confidence intervals (CIs) were calculated. Standard deviations were computed from standard errors or 95% CIs where needed. For crossover trials, the pre–post correlation coefficient (r) was calculated based on the study by West et al. [18], which reported baseline, final, and change values. The estimated

r (0.8) was then applied to all outcomes, as no other studies provided sufficient data for direct calculation. Accordingly, SDs were pooled using this correlation coefficient. A random-effects model (using the DerSimonian and Laird or REML method, depending on the software) was employed throughout due to expected clinical and methodological heterogeneity across studies. Statistical heterogeneity was quantified using the I^2 statistic, with thresholds of 25%, 50%, and 75% representing low, moderate, and high heterogeneity, respectively.

Subgroup analyses were conducted to explore potential sources of heterogeneity. The cut-off values for subgrouping were determined a priori based on clinical relevance and biological plausibility. Age was dichotomized at 45 years, a threshold commonly used in nutritional and metabolic studies to distinguish younger from middle-aged and older adults with differing cardiometabolic risk profiles [19, 20]. Body mass index (BMI) was categorized as < 30 or ≥ 30 kg/m², consistent with the World Health Organization definition of obesity [21]. Health status was classified as healthy versus individuals with metabolic syndrome or obesity, given their distinct metabolic characteristics that could influence choline metabolism and TMAO production.

To further investigate sources of heterogeneity, random-effects meta-regression analyses were performed using restricted maximum likelihood (REML) estimation. The continuous moderator variables explored included participant age, egg dosage, and duration of intervention in weeks. For each covariate, the proportion of between-study variance explained (R^2) was calculated to assess its contribution to heterogeneity. The influence of each moderator on the effect estimates was assessed based on the strength of the association, as indicated by the p-value, with values less than 0.05 suggesting a stronger relationship with the effect size.

Assessment of publication bias was conducted through multiple approaches. Funnel plots were visually inspected for asymmetry, and Egger's regression test and Begg's test were applied to statistically evaluate the presence of small-study effects. Although the number of included studies was fewer than ten for some outcomes—limiting the power of these statistical tests—they were performed cautiously due to concerns about potential publication bias. Additionally, the nonparametric trim-and-fill method was used to estimate the number and impact of potentially missing studies, helping to assess whether asymmetry might have influenced the pooled effect sizes.

NutriGRADE assessment

The certainty of evidence was assessed using the NutriGRADE scoring system, which is specifically developed for nutrition-related meta-analyses. This approach considers several domains, including study quality and risk of bias, precision of effect estimates, statistical heterogeneity, directness of the evidence, publication bias, funding bias, study design, and the magnitude of effect sizes. Each domain contributes to a total score ranging from 0 to 10 points. Based on the overall score, the certainty of evidence is categorized as high (≥ 8 points), moderate (6 to < 8), low (4 to < 6), or very low (< 4) [22]. For the present analysis, NutriGRADE ratings were independently performed by two reviewers, and disagreements were resolved by consensus.

Deviations from protocol

Two minor deviations from the original protocol should be noted. First, in addition to PubMed and Scopus, Web of Science was included as a third database to enhance the comprehensiveness of the literature search and to minimize the risk of missing relevant studies. Second, while the original protocol focused on systematic review of randomized controlled trials, meta-analysis and certainty of evidence assessment using the NutriGRADE framework were performed post hoc, given the availability and consistency of extractable quantitative data. These additions were considered to improve the robustness and interpretability of the findings.

Results

Study selection

A total of 170 records were identified through database searches. After removing 46 duplicates, 124 records remained for title and abstract screening. Of these, 91 were excluded as they did not meet the inclusion criteria based on title and abstract. The remaining 33 full-text articles were assessed for eligibility. Following full-text screening, 27 articles were excluded for not meeting the inclusion criteria (Supplementary Table 2), and 6 randomized controlled trials were included in the final qualitative and quantitative synthesis. The study selection process is illustrated in the PRISMA 2020 flow diagram (Fig. 1). For transparency and comparability, the mean levels of circulating choline, betaine, and TMAO in intervention and comparator groups from each included study are provided in Supplementary Table 3.

Characteristics of included studies

A total of six randomized controlled trials (RCTs) were included in the meta-analysis, encompassing both crossover and parallel designs, all conducted in the United States. Study characteristics are presented in Table 1. The total sample sizes ranged from 15 to 38 participants per study arm, with intervention durations of 4 to 8 weeks. Participants included adults with varying health status (healthy, overweight/obese, metabolic syndrome) across different age groups. Importantly, both

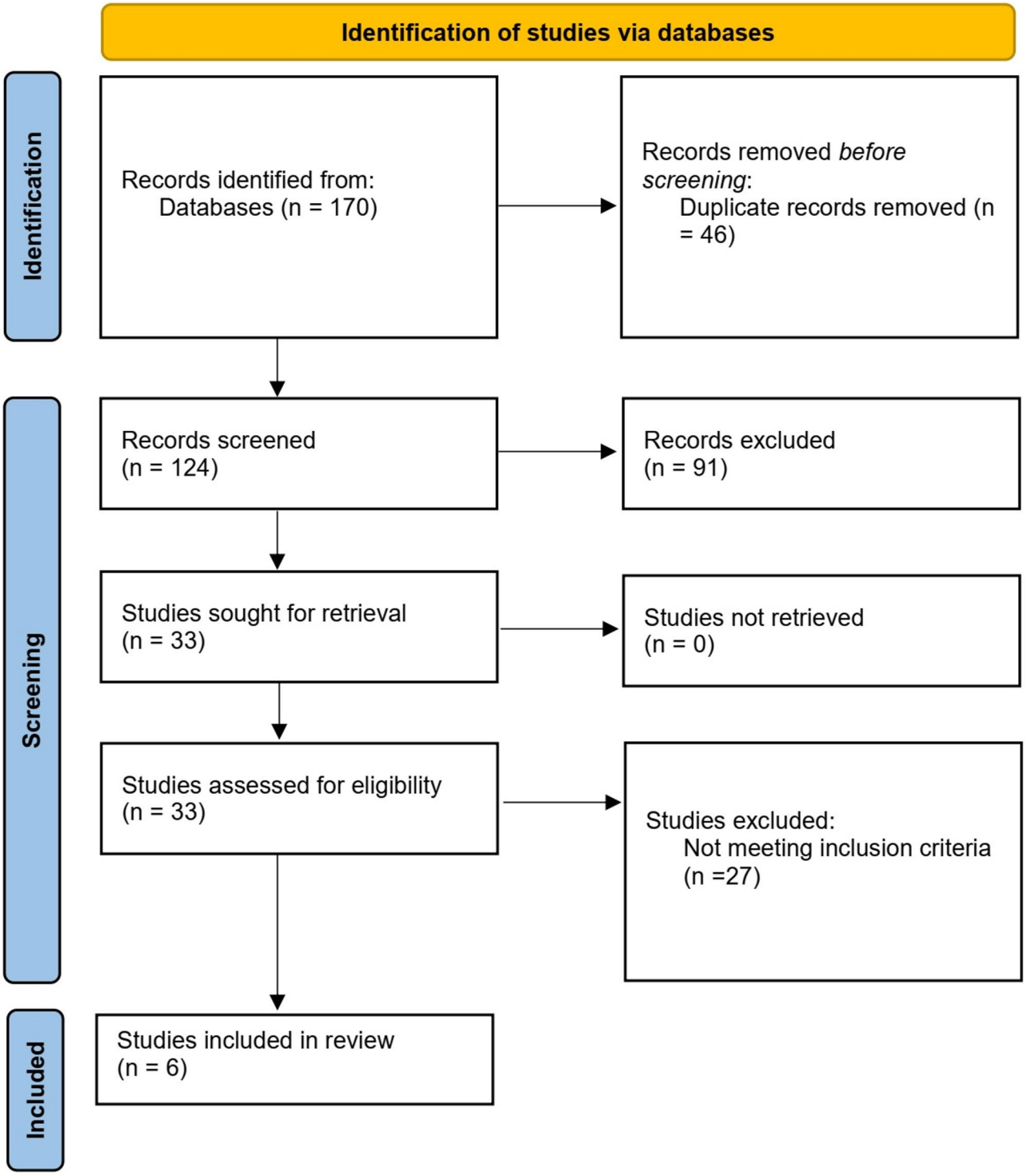


Fig. 1 PRISMA Flow Diagram

pre- and postmenopausal women were represented, ensuring diversity in sex and hormonal status among study populations.

Interventions involved the consumption of whole eggs, egg-based products, or egg components (e.g., egg whites), with daily dosages ranging from 2 to 4 eggs/day or 6 eggs/week, compared to control conditions such as choline bitartrate supplements, phosphatidylcholine, egg substitutes, or egg-free diets. In Wilcox et al. (2021), some intervention arms combined eggs with choline bitartrate

Table 1 Characteristics of included studies

Author (Year)	Country	Study Design	Population	Age (I/C)	BMI (I/C)	Sample Size (I/C)	Duration (weeks)	Intervention	Dose	Comparator	Health Status	Outcomes
DiBella et al. (2020) (1)	USA	RCT, Crossover	F/M	55.2 ± 8.9/55.2 ± 8.9	32.4 ± 4.7/32.4 ± 4.6	23 (11/12)	4	Whole eggs	3/day	Choline bitartrate supplement	Metabolic Syndrome	Choline
West et al. (2014) (2)	USA	RCT, SB, Crossover	F	35.7 ± 12.9/35.7 ± 12.9	23.7 ± 4.7/23.7 ± 4.7	15 (7/8)	8	N-3 enriched eggs or non-enriched eggs, both within a lacto-ovo-vegetarian diet	6/week	Egg-free control vegetarian diet	Women of reproductive age	Choline, Betaine, TMAO
Thomas et al. (2022 A) (3)	USA	RCT, Crossover	F/M	55.2 ± 8.9/55.2 ± 8.9	32.4 ± 4.7/32.4 ± 4.6	23 (12/11)	4	Whole eggs	3/day	Choline bitartrate supplement	Metabolic Syndrome	Choline, Betaine, TMAO
Thomas et al. (2022 B) (4)	USA	RCT, Crossover	F/M	49.3 ± 8/49.3 ± 8	34.3 ± 4.6/34.3 ± 4.6	24 (12/12)	4	Whole eggs + spinach	2/day	Egg substitute + spinach	Metabolic Syndrome	Choline, Betaine, TMAO
Zhu et al. (2020) (5)	USA	RCT, Crossover	F	57.7 ± 5.64/57.7 ± 5.64	28.3 ± 3.0/28.3 ± 3.0	20	4	Whole eggs	2/day	Yolk-free egg	Postmenopausal, overweight	Choline, Betaine, TMAO
Wilcox et al. (2021) (6)	USA	RCT, Parallel	F/M	A: 25.77 ± 4.07/28.5 ± 10.37 B: 25.77 ± 4.07/34.02 ± 15.92 C: 37.07 ± 19.48/28.5 ± 10.37 D: 30.2 ± 5.77/28.5 ± 10.37	A: 26.6 ± 6.3/25.4 ± 3.9 B: 26.6 ± 6.3/28.8 ± 5.3 C: 26.0 ± 5.7/25.4 ± 3.9 D: 30.6 ± 4.9/25.4 ± 3.9	A: 18/20 B: 18/10 C: 16/20 D: 18/20	4	A: Hardboiled eggs B: Hardboiled eggs C: Hardboiled eggs + choline bitartrate D: Egg whites + choline bitartrate	4/day	A: Choline bitartrate tablets B: Phosphatidylcholine supplements C: Choline bitartrate tablets D: Choline bitartrate tablets	Healthy volunteers	Choline, Betaine, TMAO

Data are presented as mean ± standard deviation (SD) or as number of participants (intervention/control)

Abbreviations *RCT* randomiz ed controlled trial, *SB* single-blind, *F* female, *M* male, *I/C* Intervention/Comparator, *BMI* Body Mass Index, *TMAO* Trimethylamine-N-oxide

supplementation, where the comparator groups also received choline bitartrate alone [14]. This design allowed the incremental effect of eggs to be evaluated independently of the supplement. The primary outcomes assessed across studies included circulating levels of choline, betaine, and TMAO.

Effect of egg consumption on Choline, Betaine, and TMAO levels

The meta-analysis results for the effect of egg consumption on circulating levels of choline, betaine, and TMAO are summarized in Table 2 and illustrated in Fig. 2. Overall, six unique randomized controlled trials were included. Wilcox et al. (2021) [14] was a multi-arm trial, and relevant arms were combined according to Cochrane guidance, leading to more pairwise comparisons than the number of studies in some analyses.

For choline, six randomized controlled trials (seven comparisons) were included. The pooled weighted mean difference (WMD) was 0.10 (95% CI: – 0.92 to 1.12), with a p-value of 0.847, suggesting no clear difference between intervention and control groups. Substantial heterogeneity was detected across studies (I^2 = 87.7%, Q = 47.23, $p < 0.0001$). Regarding betaine, the analysis included five studies (six comparisons). The pooled WMD was 0.21 (95% CI: – 8.50 to 8.92), with a p-value of 0.962, indicating no notable difference between groups. Heterogeneity was high (I^2 = 90.4%, Q = 50.32, $p < 0.0001$). For TMAO, five studies (six comparisons) were analyzed. The pooled WMD was – 0.08 (95% CI: – 0.47 to 0.32), with a p-value of 0.692, suggesting no clear difference between intervention and control groups. Heterogeneity was low (I^2 = 23.0%, Q = 15.05, $p = 0.0102$).

Publication bias

Potential publication bias was examined using both Egger's test and Begg's test, as well as through nonparametric trim-and-fill analysis. As shown in Table 2, there was no evidence of small-study effects for choline and TMAO, with non-significant results from both Egger's and Begg's tests. For betaine, Egger's test suggested the possibility of publication bias ($p = 0.0332$), although Begg's test did not support this ($p = 0.1329$). To further assess the potential impact of publication bias, a trim-and-fill analysis was conducted. For betaine, the analysis did not impute any missing studies, indicating that the asymmetry detected may not have meaningfully influenced the pooled effect estimate. The pooled weighted mean difference remained unchanged before and after imputation (WMD = 2.736; 95% CI: 0.090 to 5.382), under both random- and fixed-effects models. Funnel plots illustrating the distribution of effect sizes are presented in Fig. 3. The plots for choline and TMAO appeared symmetrical, while betaine showed mild asymmetry, consistent with the Egger's test result but not supported by trim-and-fill adjustment.

Sensitivity analysis

To evaluate the robustness of the meta-analysis findings, sensitivity analyses were conducted using a leave-one-out approach for each outcome (choline, betaine, and TMAO). For choline, the overall effect estimate remained stable across all iterations, with WMDs ranging narrowly and no single study unduly influencing the pooled effect (range of p-values: 0.268 to 0.991). Similarly, for betaine, exclusion of individual studies did not substantially alter the overall result, with all recalculated WMDs remaining non-significant (range of p-values: 0.404 to 0.957), indicating consistency across studies. For TMAO, the pooled estimate was also unaffected by the removal of any single study, and no shift toward statistical significance was

Table 2 Meta-analysis summary results for the effect of egg consumption on Circulating Choline, Betaine, and TMAO levels

Outcome Variables	No. of Studies	WMD (95% CI)	*P* Value	Assessment of heterogeneity		Q *P* Value	Pooling Method	Publication bias	
				I^2 (%)	Q-statistic			Egger Test *P* Value	Begg's Test *P* Value
Choline (μmol/l)	6 (1–6)*	0.10 (–0.92, 1.12)	0.847	87.7	47.23	0.0000	Random-effects (REML)	0.2581	0.0715
Betaine (μmol/l)	5 (2–6)*	0.21 (–8.50, 8.92)	0.962	90.4	50.32	0.0000	Random-effects (REML)	0.0332	0.1329
TMAO (μmol/l)	5 (2–6)*	–0.08 (–0.47, 0.32)	0.692	23.0	15.05	0.0102	Random-effects (REML)	0.2468	1.0000

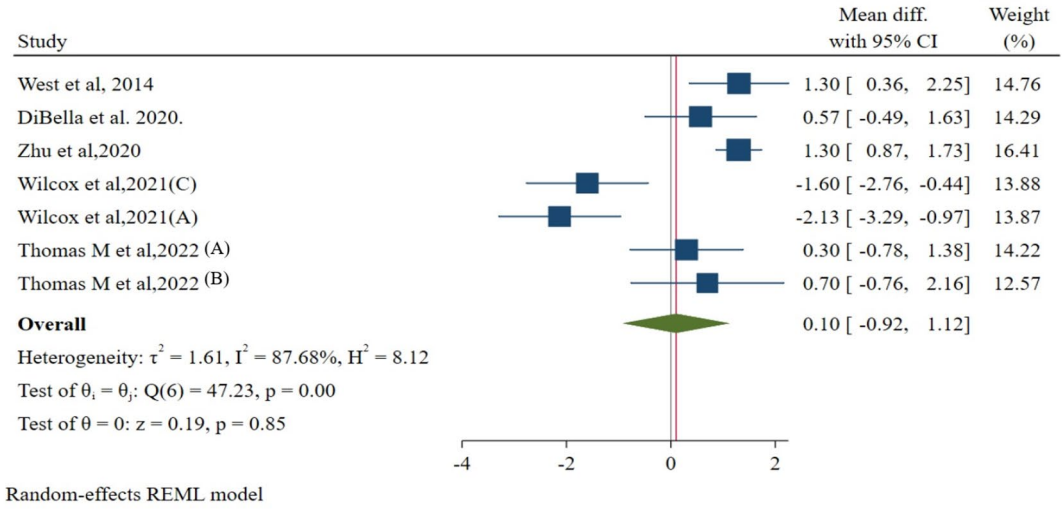
Data reported as WMD

I2 index ≥ 50% indicates moderate-to-high heterogeneity

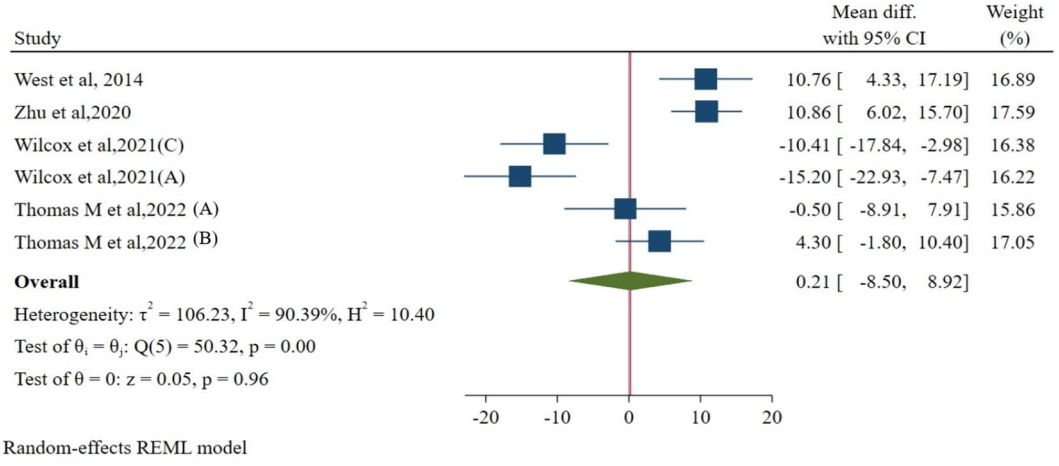
A p-value less than 0.05 was considered as evidence of a potentially meaningful association

Abbreviations: Trimethylamine N-oxide = TMAO; WMD = Weighted Mean Difference; CI = Confidence Interval; I^2 = Heterogeneity Index; REML = Restricted Maximum Likelihood

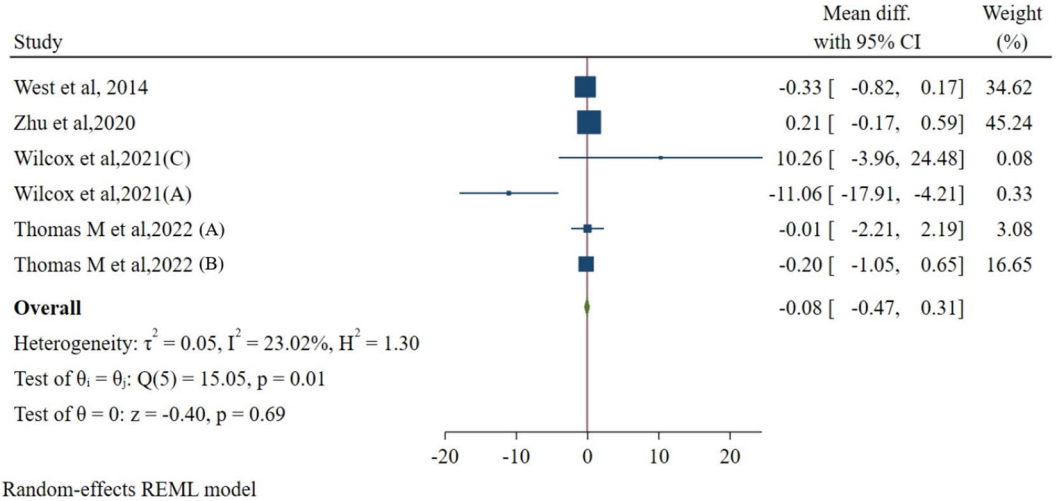
* One study (Wilcox et al., 2021) included four arms. To avoid double-counting, two arms with a common intervention and two arms with a common control were combined into two independent comparisons and analyzed separately in the meta-analysis [6]



A. Choline

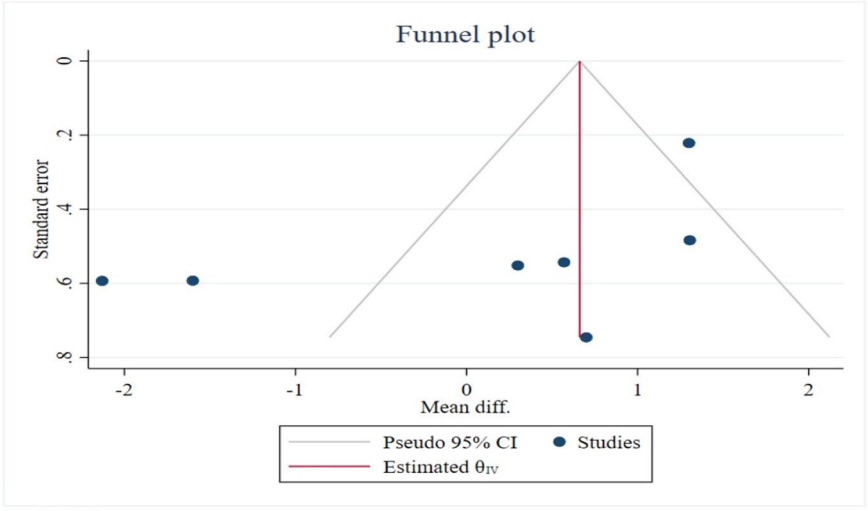


B. Betaine

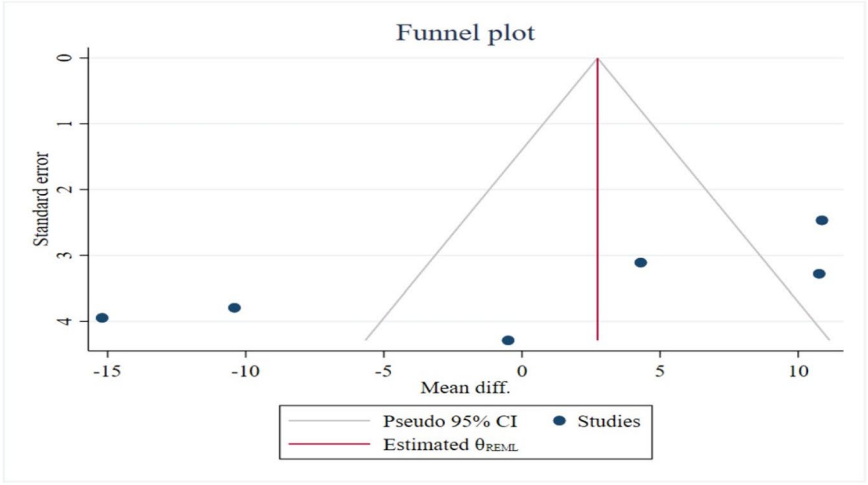


C. TMAO

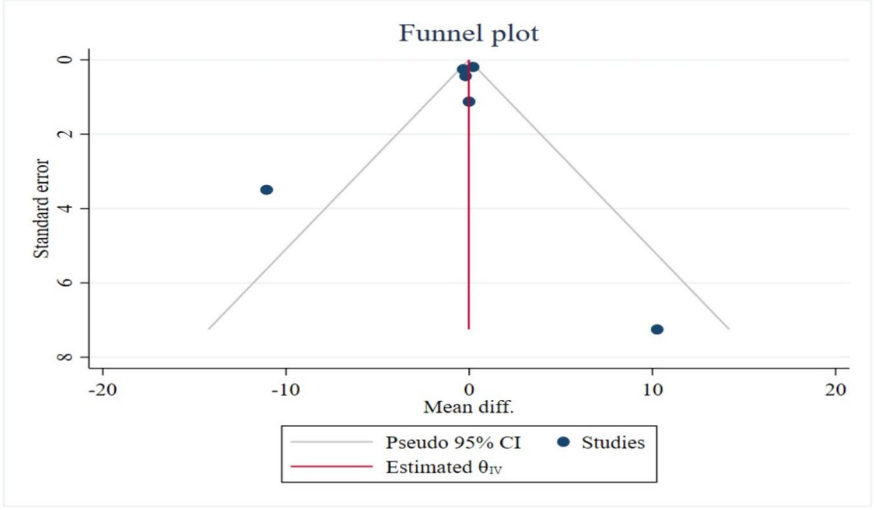
Fig. 2 Forest plot showing the weighted mean differences (WMDs) and corresponding 95% confidence intervals (CIs) for the effect of egg consumption on circulating biomarkers: **A** Choline (μmol/l), **B** Betaine (μmol/l), **C** Trimethylamine N-oxide (TMAO) (μmol/l) Wilcox et al. (2021) included four intervention arms; arms A and B, which shared a common intervention, were combined and presented as Wilcox et al. 2021 (A), and arms C and **D** which shared a common control, were combined and presented as Wilcox et al. 2021 (C), as described in the Methods



A. Choline



B. Betaine



C. TMAO

Fig. 3 Funnel plots assessing publication bias for the outcomes of choline **A** betaine **B** and Trimethylamine N-oxide (TMAO) **C**

observed (p-values: 0.142 to 0.836). These findings suggest that the results for all three biomarkers were robust and not overly dependent on any individual trial.

Subgroup analysis

To explore potential sources of heterogeneity, subgroup analyses were conducted based on age group (≤ 45 years vs. >45 years), health status (healthy vs. metabolic syndrome/obese), and BMI category (< 30 vs. ≥30 kg/m²) for each outcome biomarker: choline, betaine, and TMAO. The results are presented in Table 3. For choline, a significant effect was observed in participants aged > 45 years (mean difference: 0.88; 95% CI: 0.31 to 1.45; $p = 0.002$) and in those with metabolic syndrome or obesity (mean difference: 0.88; 95% CI: 0.31 to 1.45; $p = 0.002$). In contrast, no clear effects were observed in younger or healthy participants. Although between-group differences were not evident (Q = 2.22, $p = 0.136$), the effect appeared more pronounced in older or metabolically compromised individuals.

For betaine, no subgroup showed a clear effect. However, participants aged > 45 years and those with metabolic syndrome showed a trend toward increased levels (mean differences: 5.56 and 5.56, respectively), while the Q-tests for subgroup differences were not significant ($p > 0.05$). For TMAO, none of the subgroups showed significant changes following egg consumption. The pooled estimates were close to null across all age, health, and BMI categories. Between-group differences also did not reach statistical significance (Q range: 0.10–0.13, $p > 0.70$). Overall, no clear evidence of subgroup interactions was detected, although the findings suggest that age and metabolic status may influence the choline response to egg consumption.

Meta-regression analysis

To further investigate potential sources of heterogeneity, meta-regression analyses were conducted for each biomarker—choline, betaine, and TMAO—based on three continuous moderator variables: participant age, intervention duration, and egg dosage. The results are summarized in Table 4. For choline, age was positively associated with the effect size (coefficient = 0.07; 95% CI: 0.00 to 0.14; $p = 0.031$), explaining approximately 43.9% of between-study variance. Egg dose was also significantly associated with the outcome (coefficient = − 1.05; 95% CI: − 1.57 to − 0.53; $p < 0.001$), with higher egg doses linked to a reduction in choline levels, accounting for 83.1% of heterogeneity. Intervention duration was not a significant predictor ($p = 0.342$).

For betaine, dose again showed a strong negative association with effect size (coefficient = − 8.16; 95% CI: − 11.52 to − 4.81; $p < 0.001$), accounting for 90.1% of

Table 3 Subgroup analysis for the effect of egg consumption on Circulating Choline, Betaine, and TMAO levels

Biomarker	Subgroup	No. of Studies	Mean Difference (95% CI)	*p*-value	Q test for subgroup (Q)	Q test *p*-value
Choline	Age ≤ 45 years	3 (2, 6)*	−0.78 (−2.90, 1.33)	0.467	2.22	0.136
	Age > 45 years	4 (1, 3–5)	0.88 (0.31, 1.45)	0.002		
	Healthy	4 (2, 6)*	−0.78 (−2.90, 1.33)	0.467	2.22	0.136
	MetS or obese	4 (1, 3–5)	0.88 (0.31, 1.45)	0.002		
	BMI < 30	4 (2, 5, 6)*	−0.23 (−2.03, 1.57)	0.801	0.54	0.461
	BMI > 30	3 (1, 3, 4)	0.49 (−0.18, 1.17)	0.151		
Betaine	Age ≤ 45 years	3 (2, 6)*	−4.83 (−20.56, 10.90)	0.547	1.44	0.231
	Age > 45 years	3 (3–5)	5.56 (−0.86, 11.97)	0.090		
	Healthy	3 (2, 6)*	−4.83 (−20.56, 10.90)	0.547	1.44	0.231
	MetS or obese	3 (3–5)	5.56 (−0.86, 11.97)	0.090		
	BMI < 30	4 (2, 5, 6)*	−0.79 (−14.27, 12.70)	0.909	0.22	0.639
	BMI > 30	2 (3, 4)	2.65 (−2.29, 7.58)	0.293		
TMAO	Age ≤ 45 years	3 (2, 6)*	−1.58 (−12.25, 9.08)	0.771	0.10	0.752
	Age > 45 years	3 (3–5)	0.14 (−0.20, 0.48)	0.419		
	Healthy	3 (2, 6)*	−1.58 (−12.25, 9.08)	0.771	0.10	0.752
	MetS or obese	3 (3–5)	0.14 (−0.20, 0.48)	0.419		
	BMI < 30	4 (2, 5, 6)*	−1.36 (−7.70, 4.97)	0.673	0.13	0.715
	BMI > 30	2 (3, 4)	−0.18 (−0.97, 0.62)	0.667		

Subgroups were based on participant age (≤ 45 or > 45 years), metabolic status, and BMI category (< 30 or ≥ 30 kg/m²)

Between-group heterogeneity was assessed using the Q-test for subgroup differences

A p-value less than 0.05 was considered as evidence of a potentially meaningful association

Abbreviations: Trimethylamine N-oxide = TMAO; MetS = Metabolic Syndrome; BMI = Body Mass Index; CI = Confidence Interval; Q = Cochran's Q test for subgroup difference

* One study (Wilcox et al., 2021) included four arms. To avoid double-counting, two arms with a common intervention and two arms with a common control were combined into two independent comparisons and analyzed separately in the meta-analysis (2021) [6]

Table 4 Meta-regression analysis of factors influencing the effect of egg consumption on Circulating Choline, Betaine, and TMAO levels

Biomarker	Variable	Coefficient	95% CI	*p*-value	R^2 (%)
Choline	Age	0.07	0.00 to 0.14	**0.031**	43.9
	Duration	0.35	−0.38 to 1.08	0.342	0.0
	Dose	−1.05	−1.57 to − 0.53	**0.000**	83.1
Betaine	Age	0.55	−0.09 to 1.20	0.093	30.0
	Duration	3.17	−2.57 to 8.91	0.279	2.70
	Dose	−8.16	−11.52 to − 4.81	**0.000**	90.1
TMAO	Age	0.03	−0.00 to 0.06	0.054	100.0
	Duration	0.16	−1.68 to 2.01	0.861	0.0
	Dose	0.03	−0.71 to 0.77	0.941	0.0

Meta-regressions were conducted using a random-effects model (REML method) with continuous moderators: participant age (years), intervention duration (weeks), and egg dosage (eggs/day or per week as standardized)

Abbreviations: Trimethylamine N-oxide = TMAO; CI = Confidence Interval; R^2 = Proportion of between-study variance explained by the covariate

A p-value less than 0.05 was considered as evidence of a potentially meaningful association

the heterogeneity. A trend was observed with age (coefficient = 0.55; $p = 0.093$), but it did not reach statistical significance. Duration of intervention had no significant effect ($p = 0.279$). For TMAO, none of the variables reached conventional levels of statistical significance, although age showed a borderline association (coefficient = 0.03; $p = 0.054$), explaining 100% of observed heterogeneity. Neither duration nor dose was significantly associated with TMAO levels ($p > 0.80$). These results suggest that age and dosage may act as effect modifiers, particularly for choline and betaine, highlighting the importance of considering participant characteristics and intervention intensity in interpreting the metabolic response to egg consumption.

NutriGRADE assessment

The certainty of evidence was evaluated using the NutriGRADE tool, which is specifically recommended for nutrition meta-analyses. This system takes into account study design, risk of bias, study limitations, precision, heterogeneity, directness, publication bias, and funding bias. The results are summarized in Supplementary Table 4. For choline, the NutriGRADE score was 7.75, corresponding to moderate certainty of evidence. While all included studies were randomized controlled trials (RCTs), some exhibited methodological limitations and high heterogeneity, which led to downgrading. For betaine, the NutriGRADE score was 7.05, also reflecting moderate certainty of evidence. Although a dose–response association was observed in meta-regression analyses, downgrades were applied due to substantial heterogeneity, imprecision of estimates, and indications of publication bias. For TMAO, the NutriGRADE score was 7.25, indicating moderate certainty of evidence. Compared to the other outcomes, methodological limitations were less concerning, heterogeneity was low, and the overall evidence was considered more consistent. Overall, NutriGRADE suggests that the certainty of evidence regarding the effects of egg consumption on circulating choline, betaine, and TMAO is moderate, with the main limitations arising from heterogeneity, imprecision, and potential publication bias.

Discussion

The pooled analysis demonstrated no significant overall effect of egg consumption on circulating levels of TMAO, choline, or betaine. Subgroup and meta-regression analyses indicated that participant age and egg dosage may influence the response, particularly for choline and betaine, highlighting the importance of inter-individual and intervention-related factors. These findings provide insights into the variability of metabolic outcomes and add nuance to the ongoing debate about the cardiometabolic implications of egg consumption.

Choline

In this meta-analysis, no significant overall change in circulating choline levels was observed following egg consumption. Subgroup and meta-regression analyses, however, revealed notable trends: increases were significant in individuals aged > 45 years and those with metabolic syndrome or obesity. Interestingly, higher egg doses were associated with smaller increases in circulating choline levels. This observation should be interpreted cautiously, as evidence on choline absorption kinetics and bioavailability is limited. While one possibility is a feedback regulatory mechanism or saturation of transport processes, such explanations remain highly speculative and are not strongly supported by the current literature [23–26]. These results imply that the impact of egg-derived choline depends on metabolic status, age-related changes in hepatic/renal function, and choline homeostasis. Menopause, which reduces endogenous choline synthesis via decreased estrogen-driven PEMT (phosphatidylethanolamine N-methyltransferase) activity, may partly explain the stronger responses observed in older women [27, 28].

Choline is an essential nutrient involved in membrane integrity, methyl donation, and neurotransmitter synthesis [29]. Eggs are a rich source, mainly as phosphatidylcholine, which has greater bioavailability than synthetic salts [30]. Yet, choline metabolism is influenced by metabolic demand, gut microbiota, and genetic polymorphisms (e.g., PEMT, MTHFR, FMO3) [31, 32]. Comparable findings have been reported in previous syntheses. Sultan et al. (2024) reported short-term (≤ 4 weeks) increases in plasma choline with egg intake, albeit with substantial between-study variability—an observation that contrasts with our null overall effect yet is

compatible with the population-specific patterns we observed (e.g., stronger responses in older or metabolically compromised adults) [33]. In cohort work focused specifically on egg intake, Greenberg et al. (2021) found that higher egg consumption was initially associated with diabetes risk, but this association was attenuated after adjustment for dietary cholesterol and broader dietary factors, highlighting the challenge of disentangling egg-related nutrients when interpreting metabolic outcomes [34]. Complementing this, a meta-analysis of prospective cohorts by Meyer and Shea (2017)—which examined total dietary choline and betaine (not egg-specific exposure)—did not find clear associations with incident cardiovascular disease [35]. Taken together, these data, alongside our results, suggest that while eggs contribute meaningfully to choline intake, the net effect on circulating choline appears modest at the aggregate level and may depend on characteristics such as age, metabolic health, and dose.

Mechanistically, choline metabolism is shaped by one-carbon metabolism, insulin sensitivity, and renal excretion [36]. In metabolic syndrome, impaired choline synthesis and altered phosphatidylcholine metabolism increase reliance on dietary intake [37, 38]. With aging, reduced PEMT activity and renal clearance may further amplify dietary responses, explaining stronger effects in older or metabolically compromised individuals [39–41].

Comparisons with supplements suggest greater bioavailability from egg-derived phosphatidylcholine. Smolders et al. (2019) showed egg yolk phospholipids produced fourfold higher plasma choline AUC (Area Under the Curve) compared to choline bitartrate, likely due to lymphatic absorption and incorporation into lipoproteins [42–44]. Eggs also provide co-nutrients (e.g., lutein, vitamin E, selenium) that may enhance choline function and reduce inflammation, as seen in DiBella et al. (2020) [45]. Despite these theoretical advantages, most included RCTs—even those with relatively high egg doses—did not show substantial differences in circulating choline between groups. Several factors may explain this: (i) adequate background choline intake in controls; (ii) high inter-individual variability in choline metabolism (e.g., genetic polymorphisms, gut microbiota); (iii) circulating concentrations may not reflect tissue uptake or utilization; and (iv) insufficient study duration. Collectively, these issues likely attenuated expected contrasts and highlight the complexity of linking bioavailability to consistent clinical outcomes.

Betaine

Our meta-analysis showed no significant overall effect of egg consumption on circulating betaine, with high heterogeneity. Subgroup analyses found no consistent differences by age, health status, or BMI, though older adults and those with metabolic disorders showed a trend toward higher levels. Meta-regression revealed a strong inverse relationship between egg dose and betaine, suggesting a nonlinear effect where higher intake may actually suppress its accumulation. Betaine is an essential methyl donor in homocysteine remethylation and supports hepatic and renal function under stress [46]. It is derived from hepatic and renal oxidation of choline [47], but this conversion is tightly regulated and influenced by one-carbon metabolism, folate status, and methylation demand [48, 49]. Folate, B12, and B6 status are particularly important since they modulate remethylation pathways [50, 51]; variation in these nutrients across populations may explain the heterogeneity in responses [52, 53].

Evidence directly synthesizing the effect of egg consumption on circulating betaine is scarce. In the Women's Health Initiative, Greenberg et al. (2021) reported no independent association between dietary betaine and diabetes risk after adjusting for cholesterol, consistent with our findings of modest, context-dependent changes [34]. Similarly, a broader meta-analysis by Meyer and Shea (2017) found no link between total dietary betaine and cardiovascular disease [35]. Together, these data suggest that betaine responses to eggs are small, heterogeneous, and shaped more by co-exposures (cholesterol, folate/B-vitamins), methylation demand, and individual physiology than by egg intake alone.

Mechanistically, the inverse dose-response may reflect saturation of the choline-to-betaine pathway or greater utilization of betaine in one-carbon metabolism under high choline intake [54, 55]. Alternatively, high folate and B12 status may shift remethylation toward folate-dependent pathways, reducing betaine reliance [53, 56]. Hepatic feedback may also downregulate betaine synthesis or enhance catabolism with higher choline, blunting plasma increases [57, 58].

Trimethylamine N-oxide

The impact of egg consumption on circulating TMAO was variable, with no significant pooled effect and low statistical heterogeneity. The forest plot for TMAO may appear visually heterogeneous, which is mainly due to wide confidence intervals in the Wilcox trial [14]. However, this trial contributed minimal weight to the overall analysis, and sensitivity analyses confirmed the robustness of the null finding. Subgroup and meta-regression analyses showed no significant effects of age, BMI, health status, dose, or duration. These findings challenge the perception that eggs substantially elevate TMAO. TMAO is produced by microbial conversion of choline, carnitine, and phosphatidylcholine to trimethylamine, followed by hepatic oxidation via FMO3 [59]. Elevated TMAO has been associated with atherosclerosis, inflammation, and

thrombosis, but causality remains debated [60]. Variability in TMAO responses to eggs likely reflects gut microbiota composition (e.g., TMA-producing taxa), FMO3 polymorphisms, renal clearance, and background diet [31, 61, 62].

The findings of the present meta-analysis align with previous syntheses indicating that egg consumption does not consistently elevate circulating TMAO concentrations. For example, Sultan et al. (2024) similarly reported no significant impact of egg intake on plasma TMAO across short-term RCTs, although heterogeneity and variable gut microbiota responses were noted [33]. More broadly, Lombardo et al. (2021), in a narrative review of dietary sources of TMAO, emphasized that while fish is a strong determinant of plasma and urinary TMAO, the evidence linking eggs to elevated TMAO is inconsistent and appears highly dependent on individual microbiota profiles and possibly on food preparation methods [63]. Similarly, Kang and Zivkovic (2021) highlighted that egg intake does not consistently alter TMAO concentrations in most individuals, although they stressed the importance of inter-individual variability—particularly differences in cholesterol absorption efficiency and gut microbial capacity for TMA production—as critical modifiers of response [13].

Strengths and limitations

This study represents the first comprehensive meta-analysis synthesizing data from randomized controlled trials to evaluate the effects of egg consumption on circulating levels of choline, betaine, and TMAO—biomarkers with mechanistic relevance to cardiometabolic health. The analysis was conducted in accordance with PRISMA guidelines, with a rigorous selection process, duplicate data extraction, and sensitivity, subgroup, and meta-regression analyses to explore potential sources of heterogeneity.

However, several limitations should be considered. First, the limited number of eligible trials and small sample sizes in some studies may reduce the statistical power to detect subtle changes, particularly in subgroup analyses. Combined with the high between-study heterogeneity, these small samples increase uncertainty and reduce confidence in subgroup findings, which should be interpreted as hypothesis-generating rather than definitive. Second, variability in intervention design, including differences in egg type (e.g., whole eggs, enriched eggs, egg whites), dose, duration, and comparator arms, may have introduced heterogeneitys. In particular, comparator groups were highly heterogeneous (habitual diet, egg-free control foods, or supplemental choline forms), raising concerns about pooling across trials. Therefore, the validity of pooled estimates is limited, and results should be interpreted cautiously as reflecting relative differences between eggs and diverse comparator sources, rather than uniform intervention effects. Third, several included trials used choline bitartrate or phosphatidylcholine supplements as comparator groups. This design may attenuate the apparent contrast between intervention and control arms, as both groups received relatively high choline intake, potentially underestimating the true effect of egg consumption. Thus, the findings should be interpreted as reflecting differences between sources of choline (eggs versus supplements), rather than strictly low versus high choline intake. Fourth, some studies did not report baseline values or adjust for confounding dietary factors, such as fiber intake, which can modulate gut microbiota activity and TMAO production. Additionally, limited or inconsistent reporting on the timing of blood collection relative to egg consumption may have influenced comparability across studies, given the strong postprandial fluctuations of choline, betaine, and TMAO. Fifth, the subgroup analyses presented in this meta-analysis were exploratory and not pre-specified in the study protocol. As such, the observed associations—particularly those related to age, metabolic status, and egg dosage—should be interpreted with caution, as they may reflect spurious findings due to multiple testing or chance rather than true biological effects. Finally, due to the short duration of most trials, long-term effects of egg consumption on these biomarkers remain unclear. Moreover, as all included trials were conducted in the United States, generalizability to other populations is limited. Differences in baseline dietary patterns, habitual choline intake, and genetic variability in one-carbon metabolism across regions may influence circulating metabolite responses to eggs.

Recommendations for future research

Future clinical trials should address current gaps by including larger and more diverse populations, particularly those with different gut microbiota compositions and chronic health conditions. Careful selection of comparator groups is essential to isolate the effects of egg consumption from other dietary factors. Longer intervention durations are needed to assess sustained metabolic impacts. Standardizing egg dose and form, along with controlling background dietary components like fiber and polyphenols, will improve comparability across studies. Consistent reporting of baseline biomarker levels and time-course responses will better capture individual variability. Future research should also explore gene–diet–microbiome interactions affecting choline metabolism, such as FMO3 polymorphisms and PEMT activity. Combining individual participant data meta-analyses with gut microbiome sequencing could provide mechanistic insights into why responses to eggs and dietary choline vary across individuals.

Conclusion

In conclusion, this meta-analysis indicates no clear effect of egg consumption on circulating choline, betaine, or TMAO levels. Subgroup and dose-response analyses suggest that individual and intervention-related factors may influence these outcomes. Based on the NutriGRADE assessment, the certainty of evidence is moderate for choline, betaine, and TMAO, with downgrades mainly due to imprecision and heterogeneity. Therefore, the findings should be interpreted with caution. Eggs can still be considered part of a balanced diet. Further robust studies are needed to clarify the interactions between egg-derived nutrients, host metabolism, and gut microbiota. Modest increases in total and LDL cholesterol at higher intakes have been reported, but these often occur alongside improvements in HDL cholesterol and lipoprotein particle size, indicating a context-dependent effect on lipid metabolism [64–67].

Abbreviations

AUC	Area Under the Curve
B6	Vitamin B6
B9	Vitamin B9
B12	Vitamin B12
BMI	Body Mass Index
CI	Confidence Interval
CVD	Cardiovascular Disease
DMG	Dimethylglycine
FMO3	Flavin-Containing Monooxygenase 3
GRADE	Grading of Recommendations Assessment, Development, and Evaluation
HDL	High-Density Lipoprotein
I^2	Heterogeneity Statistic (I-squared)
LDL	Low-Density Lipoprotein
MEDLINE	Medical Literature Analysis and Retrieval System Online
MTHFR	Methylenetetrahydrofolate Reductase
PEMT	Phosphatidylethanolamine N-Methyltransferase
PICO	Population, Intervention, Comparator, Outcome
PRESS	Peer Review of Electronic Search Strategies
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PROSPERO	International Prospective Register of Systematic Reviews
Q	Cochrane's Q Statistic
r	Correlation Coefficient
RCTs	Randomized Controlled Trials
REML	Restricted Maximum Likelihood
RevMan	Review Manager
RoB 2	Risk of Bias 2.0
R^2	R-squared (proportion of between-study variance explained)
SD	Standard Deviation
TMA	Trimethylamine
TMAO	Trimethylamine N-Oxide
WMD	Weighted Mean Difference
WOS	Web of Science

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12937-025-01227-0.

Supplementary material 1.

Supplementary material 2.

Supplementary material 3.

Supplementary material 4.

Acknowledgements

The authors thank and appreciate all the professors from the School of Public Health, Iran University of Medical Sciences.

Authors' contributions

NA supervised the overall work. AZ, NT, and HH were responsible for data extraction, screening, and initial review of the studies. ARA performed data analysis and interpretation. SHD contributed to the project design, conducted screening, drafted the manuscript, and supervised the writing process. All authors read and approved the final manuscript.

Funding

This study received no financial support or funding.

Data availability

The datasets used and analyzed during the current study available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]Department of Nutrition, School of Public Health, Iran University of Medical Sciences, Hemmat Highway, Tehran, Iran
[2]Department of Nutrition, School of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[3]Research Center for Food Hygiene and Safety, School of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[4]Student Research Committee, Urmia University of Medical Sciences, Urmia, Iran
[5]Isfahan Endocrine and Metabolism Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

Received: 29 June 2025 / Accepted: 26 September 2025
Published online: 27 October 2025

References

1. Réhault-Godbert S, Guyot N, Nys Y. The golden egg: nutritional value, bioactivities, and emerging benefits for human health. Nutrients. 2019;11(3):684.
2. Usturoi MG, Rațu RN, Crivei IC, Veleșcu ID, Usturoi A, Stoica F, et al. Unlocking the power of eggs: nutritional insights, bioactive compounds, and the advantages of Omega-3 and Omega-6 enriched varieties. Agriculture. 2025;15(3):242.
3. Kuang H, Yang F, Zhang Y, Wang T, Chen G. The impact of egg nutrient composition and its consumption on cholesterol homeostasis. Cholesterol. 2018;2018(1):6303810.
4. Zhen J, Zhou Z, He M, Han H-X, Lv E-H, Wen P-B, et al. The gut microbial metabolite trimethylamine N-oxide and cardiovascular diseases. Front Endocrinol. 2023;14:1085041.
5. Caradonna E, Abate F, Schiano E, Paparella F, Ferrara F, Vanoli E, et al. Trimethylamine-N-oxide (TMAO) as a rising-star metabolite: implications for human health. Metabolites. 2025;15(4):220.
6. Zhou Y, Zhang Y, Jin S, Lv J, Li M, Feng N. The gut microbiota derived metabolite trimethylamine N-oxide: its important role in cancer and other diseases. Biomed Pharmacother. 2024;177:117031.
7. Li X, Hong J, Wang Y, Pei M, Wang L, Gong Z. Trimethylamine-N-oxide pathway: a potential target for the treatment of MAFLD. Front Mol Biosci. 2021;8:733507.
8. Hou CY, Chen YW, Hazeena SH, Tain YL, Hsieh CW, Chen DQ, et al. Cardiovascular risk of dietary trimethylamine oxide precursors and the therapeutic potential of resveratrol and its derivatives. FEBS Open Bio. 2024;14(3):358–79.

9. Wang B, Qiu J, Lian J, Yang X, Zhou J. Gut metabolite trimethylamine-N-oxide in atherosclerosis: from mechanism to therapy. Front Cardiovasc Med. 2021;8:723886.
10. Hazen SL, Brown JM. Eggs as a dietary source for gut microbial production of trimethylamine-N-oxide123. Am J Clin Nutr. 2014;100(3):741–3.
11. Arias N, Arboleya S, Allison J, Kaliszewska A, Higarza SG, Gueimonde M, et al. The relationship between choline bioavailability from diet, intestinal microbiota composition, and its modulation of human diseases. Nutrients. 2020;12(8):2340.
12. Miller CA, Corbin KD, Da Costa K-A, Zhang S, Zhao X, Galanko JA, et al. Effect of egg ingestion on trimethylamine-N-oxide production in humans: a randomized, controlled, dose-response study. Am J Clin Nutr. 2014;100(3):778–86.
13. Kang JW, Zivkovic AM. Are eggs good again? A precision nutrition perspective on the effects of eggs on cardiovascular risk, taking into account plasma lipid profiles and TMAO. J Nutr Biochem. 2022;100:108906.
14. Wilcox J, Skye SM, Graham B, Zabell A, Li XS, Li L, et al. Dietary choline Supplements, but not Eggs, Raise fasting TMAO levels in participants with normal renal function: A randomized clinical trial. Am J Med. 2021;134(9):1160–e93.
15. Ilyas A, Wijayasinghe YS, Khan I, El Samaloty NM, Adnan M, Dar TA, et al. Implications of trimethylamine N-oxide (TMAO) and betaine in human health: beyond being osmoprotective compounds. Front Mol Biosci. 2022;9:964624.
16. Yu M, Liu G, Chen W, Qiu Y, You N, Chen S, et al. Choline metabolism in ischemic stroke: an underappreciated two-edged sword. Pharmacol Res. 2025. https://doi.org/10.1016/j.phrs.2025.107685.
17. Eslami M, Alibabaei F, Babaeizad A, Banihashemian SZ, Mazandarani M, Hoseini A, et al. The importance of gut microbiota on choline metabolism in neurodegenerative diseases. Biomolecules. 2024;14(11):1345.
18. West AA, Shih Y, Wang W, Oda K, Jaceldo-Siegl K, Sabaté J, et al. Egg n-3 fatty acid composition modulates biomarkers of choline metabolism in free-living lacto-ovo-vegetarian women of reproductive age. J Acad Nutr Diet. 2014;114(10):1594–600.
19. Howden EJ, Sarma S, Lawley JS, Opondo M, Cornwell W, Stoller D, et al. Reversing the cardiac effects of sedentary aging in middle age—a randomized controlled trial: implications for heart failure prevention. Circulation. 2018;137(15):1549–60.
20. Auro K, Joensuu A, Fischer K, Kettunen J, Salo P, Mattsson H, et al. A metabolic view on menopause and ageing. Nat Commun. 2014;5(1):4708.
21. Yao Z, Dardari ZA, Razavi AC, Silver N, Jelwan Y, Erhabor J, et al. Prevalence of clinical obesity versus BMI-defined obesity among US adults: a cohort study. The Lancet Diabetes & Endocrinology; 2025.
22. Schwingshackl L, Knüppel S, Schwedhelm C, Hoffmann G, Missbach B, Stelmach-Mardas M, et al. Perspective: nutrigrade: a scoring system to assess and judge the meta-evidence of randomized controlled trials and cohort studies in nutrition research. Adv Nutr. 2016;7(6):994–1004.
23. Bader S, Klein J, Diener M. Choline acetyltransferase and organic cation transporters are responsible for synthesis and propionate-induced release of acetylcholine in colon epithelium. Eur J Pharmacol. 2014;733:23–33.
24. Nakata T, Matsui T, Kobayashi K, Kobayashi Y, Anzai N. Organic cation transporter 2 (SLC22A2), a low-affinity and high-capacity choline transporter, is preferentially enriched on synaptic vesicles in cholinergic neurons. Neuroscience. 2013;252:212–21.
25. Thomas MS, Puglisi M, Malysheva O, Caudill MA, Sholola M, Cooperstone JL, et al. Eggs improve plasma biomarkers in patients with metabolic syndrome following a plant-based diet—a randomized crossover study. Nutrients. 2022;14(10):2138.
26. Thomas MS, DiBella M, Blesso CN, Malysheva O, Caudill M, Sholola M, et al. Comparison between egg intake versus choline supplementation on gut microbiota and plasma carotenoids in subjects with metabolic syndrome. Nutrients. 2022;14(6):1179.
27. Skorupa A, Poński M, Ciszek M, Cichoń B, Klimek M, Witek A, et al. Grading of endometrial cancer using 1H HR-MAS NMR-based metabolomics. Sci Rep. 2021;11(1):18160.
28. Fischer LM, da Costa K-A, Kwock L, Galanko J, Zeisel SH. Dietary choline requirements of women: effects of estrogen and genetic variation. Am J Clin Nutr. 2010;92(5):1113–9.
29. Krzysztof Blusztajn J, Mellott J. T. Choline nutrition programs brain development via DNA and histone methylation. Central nervous system agents in medicinal chemistry (Formerly current medicinal chemistry-Central nervous system agents). 2012;12(2):82–94.
30. Smolders L, de Wit NJ, Balvers MG, Obeid R, Vissers MM, Esser D. Natural choline from egg yolk phospholipids is more efficiently absorbed compared with choline bitartrate; outcomes of a randomized trial in healthy adults. Nutrients. 2019;11(11):2758.
31. Romano KA, Vivas EI, Amador-Noguez D, Rey FE. Intestinal microbiota composition modulates choline bioavailability from diet and accumulation of the proatherogenic metabolite trimethylamine-N-oxide. MBio. 2015;6(2):02481–14. https://doi.org/10.1128/mbio.
32. Smallwood T, Allayee H, Bennett BJ. Choline metabolites: gene by diet interactions. Curr Opin Lipidol. 2016;27(1):33–9.
33. Sultan N, Tuck CJ, Cheng E, Kellow NJ, Biesiekierski JR. The impact of egg consumption on gastrointestinal health: a systematic literature review and meta-analysis. Nutrients. 2025;17(13):2059.
34. Greenberg JA, Jiang X, Tinker LF, Snetselaar LG, Saquib N, Shadyab AH. Eggs, dietary cholesterol, choline, betaine, and diabetes risk in the women's health initiative: a prospective analysis. Am J Clin Nutr. 2021;114(1):368–77.
35. Meyer KA, Shea JW. Dietary choline and betaine and risk of CVD: a systematic review and meta-analysis of prospective studies. Nutrients. 2017;9(7):711.
36. Chen X, Qiu W, Ma X, Ren L, Feng M, Hu S, et al. Roles and mechanisms of choline metabolism in nonalcoholic fatty liver disease and cancers. Frontiers in Bioscience-Landmark. 2024;29(5):182.
37. Corbin KD, Zeisel SH. Choline metabolism provides novel insights into nonalcoholic fatty liver disease and its progression. Curr Opin Gastroenterol. 2012;28(2):159–65.
38. Vallianou NG, Kounatidis D, Psallida S, Panagopoulos F, Stratigou T, Geladari E, et al. The interplay between dietary choline and cardiometabolic disorders: a review of current evidence. Curr Nutr Rep. 2024;13(2):152–65.
39. Watkins SM, Zhu X, Zeisel SH. Phosphatidylethanolamine-N-methyltransferase activity and dietary choline regulate liver-plasma lipid flux and essential fatty acid metabolism in mice. J Nutr. 2003;133(11):3386–91.
40. Zhou Q, Zhang F, Kerbl-Knapp J, Korbelius M, Kuentzel KB, Vujić N, et al. Phosphatidylethanolamine n-methyltransferase knockout modulates metabolic changes in aging mice. Biomolecules. 2022;12(9):1270.
41. Korbecki J, Bosiacki M, Kupnicka P, Barczak K, Ziętek P, Chlubek D, et al. Biochemistry and diseases related to the interconversion of phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine. Int J Mol Sci. 2024;25(19):10745.
42. Smolders L, DeWit NJW, Balvers MGJ, Obeid R, Vissers MMM, Esser D. Natural choline from egg yolk phospholipids is more efficiently absorbed compared with choline bitartrate; outcomes of a randomized trial in healthy adults. Nutrients. 2019. https://doi.org/10.3390/nu11112758.
43. Blesso CN. Egg phospholipids and cardiovascular health. Nutrients. 2015;7(4):2731–47.
44. Ulus RWMCI. 18 Choline and Its Products Acetylcholine and Phosphatidylcholine. 2009.
45. DiBella M, Thomas MS, Alyousef H, Millar C, Blesso C, Malysheva O, et al. Choline intake as supplement or as a component of eggs increases plasma choline and reduces interleukin-6 without modifying plasma cholesterol in participants with metabolic syndrome. Nutrients. 2020. https://doi.org/10.3390/nu12103120.
46. Arumugam MK, Paal MC, Donohue TM, Ganesan M, Osna NA, Kharbanda KK. Beneficial effects of betaine: a comprehensive review. Biology. 2021;10(6):456.
47. Dobrijević D, Pastor K, Nastić N, Özogul F, Krulj J, Kokić B, et al. Betaine as a functional ingredient: metabolism, health-promoting attributes, food sources, applications and analysis methods. Molecules. 2023;28(12):4824.
48. Ducker GS, Rabinowitz JD. One-carbon metabolism in health and disease. Cell Metab. 2017;25(1):27–42.
49. Fernàndez-Roig S, Cavalle-Busquets P, Fernandez-Ballart JD, Ballesteros M, Berrocal-Zaragoza MI, Salat-Batlle J, et al. Low folate status enhances pregnancy changes in plasma betaine and dimethylglycine concentrations and the association between betaine and homocysteine123. Am J Clin Nutr. 2013;97(6):1252–9.
50. Sobral AF, Cunha A, Silva V, Gil-Martins E, Silva R, Barbosa DJ. Unveiling the therapeutic potential of folate-dependent one-carbon metabolism in cancer and neurodegeneration. Int J Mol Sci. 2024;25(17):9339.
51. Franco CN, Seabrook LJ, Nguyen ST, Leonard JT, Albrecht LV. Simplifying the B complex: how vitamins B6 and B9 modulate one carbon metabolism in cancer and beyond. Metabolites. 2022;12(10):961.
52. Huang J, Khatun P, Xiong Y, Liu B, Zhao Y, Lyu Q. Intakes of folate, vitamin B6, and vitamin B12 and cardiovascular disease risk: a national population-based cross-sectional study. Front Cardiovasc Med. 2023;10:1237103.
53. Dang S, Jain A, Dhanda G, Bhattacharya N, Bhattacharya A, Senapati S. One carbon metabolism and its implication in health and immune functions. Cell Biochem Funct. 2024;42(1):e3926.

54. Zeisel SH. Metabolic crosstalk between choline/1-carbon metabolism and energy homeostasis. Clin Chem Lab Med. 2013;51(3):467–75.
55. Sivanesan S, Taylor A, Zhang J, Bakovic M. Betaine and choline improve lipid homeostasis in obesity by participation in mitochondrial oxidative demethylation. Front Nutr. 2018;5:61.
56. Yeroshkina K, Rossokha Z, Fishchuk L, Gorovenko N. Betaine consumption as a new clinical approach to treatment and prophylaxis of folate-related pathologies. Nutr Rev. 2023;81(6):716–26.
57. Jang H, Lim H, Park K-H, Park S, Lee H-J. Changes in plasma choline and the betaine-to-choline ratio in response to 6-month lifestyle intervention are associated with the changes of lipid profiles and intestinal microbiota: the ICAAN study. Nutrients. 2021;13(11):4006.
58. Wargo MJ. Homeostasis and catabolism of choline and Glycine betaine: lessons from *Pseudomonas aeruginosa*. Appl Environ Microbiol. 2013;79(7):2112–20.
59. Velasquez MT, Ramezani A, Manal A, Raj DS. Trimethylamine N-oxide: the good, the bad and the unknown. Toxins. 2016;8(11):326.
60. Canyelles M, Borràs C, Rotllan N, Tondo M, Escolà-Gil JC, Blanco-Vaca F. Gut microbiota-derived TMAO: a causal factor promoting atherosclerotic cardiovascular disease? Int J Mol Sci. 2023;24(3):1940.
61. Cho CE, Taesuwan S, Malysheva OV, Bender E, Tulchinsky NF, Yan J, et al. Trimethylamine-N-oxide (TMAO) response to animal source foods varies among healthy young men and is influenced by their gut microbiota composition: a randomized controlled trial. Mol Nutr Food Res. 2017;61(1):1600324.
62. Tacconi E, Palma G, De Biase D, Luciano A, Barbieri M, de Nigris F, et al. Microbiota effect on trimethylamine N-oxide production: from cancer to fitness—a practical preventing recommendation and therapies. Nutrients. 2023;15(3):563.
63. Lombardo M, Aulisa G, Marcon D, Rizzo G, Tarsisano MG, Di Renzo L, et al. Association of urinary and plasma levels of trimethylamine N-oxide (TMAO) with foods. Nutrients. 2021;13(5):1426.
64. Rouhani MH, Rashidi-Pourfard N, Salehi-Abargouei A, Karimi M, Haghighatdoost F. Effects of egg consumption on blood lipids: a systematic review and meta-analysis of randomized clinical trials. J Am Coll Nutr. 2018;37(2):99–110.
65. Blesso CN, Fernandez ML. Dietary cholesterol, serum lipids, and heart disease: are eggs working for or against you? Nutrients. 2018;10(4):426.
66. Andersen CJ, Blesso CN, Lee J, Barona J, Shah D, Thomas MJ, et al. Egg consumption modulates HDL lipid composition and increases the cholesterol-accepting capacity of serum in metabolic syndrome. Lipids. 2013;48(6):557–67.
67. DiMarco DM, Norris GH, Millar CL, Blesso CN, Fernandez ML. Intake of up to 3 eggs per day is associated with changes in HDL function and increased plasma antioxidants in healthy, young adults. J Nutr. 2017;147(3):323–9.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.