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REVIEW

Novel findings of the association between gut microbiota-derived metabolite trimethylamine N-oxide and inflammation: results from a systematic review and dose-response meta-analysis

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ABSTRACT

The gut microbiota-derived metabolite trimethylamine *N*-oxide (TMAO) has been regarded as one of the potent risk factors of cardiovascular events and diabetes. However, its association with possible inflammatory mediators has not been revealed yet. In the current meta-analysis, we quantitatively summarized the results of studies regarding the association between TMAO and inflammation. Electronic databases including PubMed, ProQuest, Scopus, and Embase were systematically searched and a total of 586 manuscripts were retrieved. After removing 223 duplicates, 363 manuscripts were reviewed. All of the studies regarding the association between TMAO and inflammatory factors were included in the systematic review and eligible studies were included in to the meta-analysis. Accordingly, 13,783 number of participants were included and the results showed that being in the highest category of TMAO was associated with 0.27 mg/L (weighted mean difference: 0.268; 95% confidence interval [CI]: 0.058–0.479; $p = 0.013$) increase in CRP concentrations compared with lowest category. The results of subgrouping and meta-regression revealed the location, CRP sample source, disease status, male percent, proportion of diabetes and smoking as the source of heterogeneity. Moreover, the dose-response meta-analysis revealed a non-linear association between increased TMAO concentrations and increased CRP concentrations (p for nonlinearity = 0.015). To our knowledge, this is first dose-response meta-analysis that summarized the results of studies about the association between circulating TMAO concentrations and inflammation risk.

KEYWORDS

C-reactive-protein; dose-response meta-analysis; gut microbiota metabolite; inflammation; risk factor; trimethylamine *N*-oxide (TMAO)

Introduction

Trimethylamine *N*-oxide (TMAO) is a metabolite produced by gut microbiota and hepatic flavin-containing monooxygenase 3 (FMO3) from trimethylamine containing nutrients, such as carnitine, phosphatidylcholine and choline (Mente et al. 2015; Mafune et al. 2016; Gruppen et al. 2017). These metabolites are abundant in egg yolk, milk, organ and muscle meats and fish as typical foods of western diet (Koeth et al. 2013; Hazen and Brown 2014). Recently, several studies have revealed the possible role of TMAO in chronic disease including cardiovascular disease and mortality (Senthong et al. 2016; Gruppen et al. 2017; Haghikia et al. 2018; Svingen et al. 2018) diabetes (Shan et al. 2017) chronic kidney disease (Mafune et al. 2016; Stubbs et al. 2019), obesity (Dehghan et al. 2020), and metabolic syndrome (Barrea et al. 2018). In the study by Mente et al (2015) in a cross-sectional study of 1286 Canadians, being in highest quintile of serum TMAO was associated with increased prevalent cardiovascular disease (CVD) after adjustment for potential confounders (odds ratio: 9.33; 95%

confidence interval [CI]: 1.88–46.37; $p = 0.006$). Other study by Randrianarisoa et al (2016) reported that increased serum TMAO levels were associated with increased carotid intima-media thickness and lifestyle intervention reduced it specifically in subjects with the highest reduction in serum TMAO. The mechanistic pathways involved in the association between TMAO and chronic non-communicable disease are not well established; increased cholesterol accumulation in macrophages and in foam cells of artery walls (Wang et al. 2011) and increased human platelet hyper-responsiveness and the potential of thrombosis (Zhu et al. 2016) has been suggested as two possible underlying mechanisms. Inflammation plays a central key role in the pathogenesis of cardiovascular events and there are numerous therapeutic approaches focusing on reducing inflammation for CVD treatment (Golia et al. 2014; Ruparelia et al. 2017; Farhangi and Najafi 2018; Farhangi et al. 2019); numerous inflammatory mediators are involved in this pathway including C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor (TNF)- α and are potent predictors

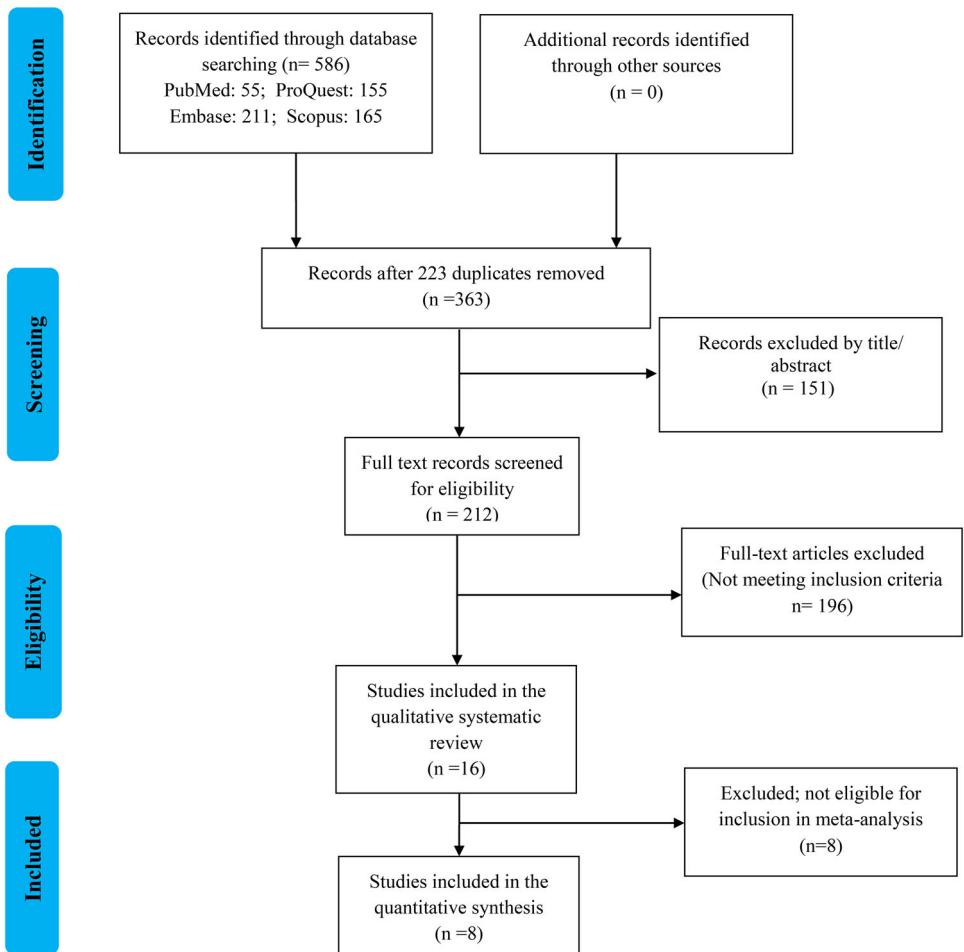


Figure 1. Flowchart of the literature search and study selection process.

of CVD events and mortality (Tuomisto et al. 2006; Tousoulis et al. 2016). Increased CRP, TNF- α , and IL-6 concentrations is associated with the degree of coronary artery syndrome and serve as a potential biomarker in prediction of acute myocardial infarction (Guo, Hao, and Tang 2018). Recently, it has been suggested that the role of TMAO in CVD is established by triggering the inflammatory process; several human and animal models revealed an increase in the expression of pro-inflammatory cytokines when plasma TMAO levels are elevated (Sun et al. 2016; Chen, Zheng, et al. 2017; Chen, Zhu, et al. 2017; Yue et al. 2017; Coras et al. 2019). Rohrmann et al. (2016) described a link between low grade inflammation and plasma TMAO concentrations. Increased plasma concentration of TMAO was associated with an increased expression of CRP, TNF- α , and IL-6. More interestingly, some of these studies revealed a dose-dependent association between TMAO and inflammation (Saco et al. 2014; Boini et al. 2017). It has been suggested that in the liver, TMAO decreases the bile acid pool and lowers the expression of key bile acid synthesis and transport proteins (Koeth et al. 2013), and reduced bile acid production is

linked to systemic inflammation (Sipka and Bruckner 2014). Most of the studies evaluating the link between TMAO and cardiovascular disease are focusing on the role of TMAO and CVD events or outcomes like mortality. However, from etiologic point of view, it is important to elucidate whether these associations are established via the independent mechanistic pathways. In the current systematic review and meta-analysis, we summarized the role of TMAO in increasing inflammatory parameters in either a two-class and dose-response synthesis.

Methods and materials

The current study was performed according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Moher et al. 2009). The completed PRISMA checklist is provided in the Supporting Information (Supplemental Table 1). The 12-item PRISMA extension checklist was also used to write the Abstract (Beller et al. 2013). The protocol of the current study has been registered in the International Prospective Register of Systematic Reviews (PROSPERO) with the identification number of

Table 1. The characteristics of the studies included in the systematic review and meta-analysis of the association between TMAO and inflammation.

Journal/ first author	Year/country	Disease status	Total no. of participants	No. of categories/no. each group	Design	Sample source	Follow-up period (y)	TMAO μmol/L	Age range (y)	Male (%)	Main results	Adjustments
<i>Journal of the American Heart Association/Yu et al. (2019)</i>	2019/China	Patients with incident CHD	275 case 275 control	4/236	Nested case-control	Plasma TMAO	—	1.43–42.31	40–74	46	No difference in plasma hs-CRP between TMAO quartiles. Significant positive associations between TMAO level and high hs-CRP ($\beta = 0.19$; $p = 0.03$) while no association between TMAO level and hs-CRP	Age, sex, family relationships, and study site
<i>Scientific Reports/Chou et al. (2019)</i>	2019/Taiwan	Patients with stable angina	81	—	Cohort	Plasma TMAO	1.5	3.3 (2.3–7.6)	≥18	69	TMAO concentration was positively correlated with the hs-CRP ($r = 0.276$; $p = 0.013$) and IL-1 β ($r = 0.332$; $p = 0.003$)	—
<i>International Journal of Cardiology/Svartengen et al. (2018)</i>	2018/participants of WNCAC	Patients with suspected stable angina	4141	4/1035	Cohort	Plasma TMAO	7.3	9.25 (2.2–23.5)	51–73	72	Significantly higher CRP levels in highest versus lowest TMAO quartile ($p = 0.03$)	—
<i>International Journal of Cardiology/Svartengen et al. (2018)</i>	2018/participants of HJUSK Study	Community-based elderly	3143	4/786	Cohort	Plasma TMAO	10.8	8.82 (2.6–31.6)	72 (71–73)	43	No significant difference in serum CRP between highest and lowest TMAO quartiles ($p = 0.72$)	—
<i>International Journal of Cardiology/Liu et al. (2018)</i>	2018/China	Patients with CAD	90	2/45	Cross-sectional	Plasma TMAO	—	Median: 114.73 (78.43, 182.7)	57.9±9.7	66	No significant difference in CRP levels in highest versus lowest group ($p = 0.073$).	Age, BMI, and WC
<i>Hormone Molecular Biology and Clinical Investigation/Lent-Schachet et al. (2018)</i>	2018/USA	Patients with MetS	50	—	Case-control	Urine TMAO	—	NR	24–72	20	TMAO significantly correlated with IL-6 ($r = 0.43$; p -value = 0.003), while no significant association between TMAO and hs-CRP, TNF- α , IL-8 was reported	—
<i>Arteriosclerosis, Thrombosis, and Vascular Biology/Haghikia et al. (2018)</i>	2018/Germany	Patients with incident stroke	78	4/20	Cohort	Plasma TMAO	1	NR	59±14	69	No significant difference in CRP levels in highest versus lowest quartile ($p = 0.09$)	Sex, age, HTN, T ₂ DM, LDL, eGFR, stroke severity, stroke, LDL, Lp(a), PAD, and history of CAD or MI
<i>Arteriosclerosis, Thrombosis, and Vascular Biology/Haghikia et al. (2018)</i>	2018/Germany	Patients with incident stroke	593	4/148	Cohort	Plasma TMAO	1	2.67–122	67±13	61	No significant difference in CRP levels in highest versus lowest quartile ($p = 0.30$)	Sex, age, HTN, T ₂ DM, LDL, eGFR, stroke severity, stroke, LDL, Lp(a), PAD, and history of CAD or MI
<i>Clinical Chemistry/Tang et al. (2017)</i>	2017/USA	Patients with T2DM	1216	3 /401	Cohort	Plasma TMAO	3–5	4.4 (2.8–7)	64.4±10.2	58	No significant difference in CRP levels in highest versus lowest quartile ($p = 0.15$)	Age, sex, history of CVD, SBP, LDL, HDL, smoking, hsCRP, Hb A1c, eGFR, BMI, and history of HF
<i>Journal of Nutrition & Intermediary Metabolism/</i>	2017/UK	Community-based adults	944	4/236	Cross-sectional	Plasma TMAO	—	Median in groups 1.43–0.47	First quartile: 45±14 Highest quartile: 51±17	48.59	No significant difference in CRP levels in highest versus lowest quartile ($p = 0.22$)	Age, sex, family relationships, and study site

(continued)



Table 1. Continued.

Journal/ first author	Year/country	Disease status	Total no. of participants	No. of categories/no. each group	Design	Sample source	Follow-up period (y)	TMAO $\mu\text{mol/L}$	Age range (y)	Male (%)	Main results	Adjustments
Aslibekyan et al. (2017) <i>Journal of the American Society of Nephrology/</i> Stubbs et al. (2016) <i>The Journal of Nutrition/</i> Rohmann et al. (2016)	2015/USA 2017/Germany	Patients with CKD Community-based adults	220 271	3/73–74 4/67–68	Cohort Cross-sectional	Serum TMAO Plasma TMAO	4 —	0.63–163.03 1.44–4.25	69.76 ± 10.3 Male: 50 (37–63) Female: 44 (36–59)	42.7	No significant association of CRP tertiles with TMAO. No significant difference in CRP levels in highest versus lowest quartile ($p = 0.67$) Participants in the highest quartile of TMAO had higher plasma values of TNF- α than lowest quartile (p -trend < 0.05), no differences in plasma concentrations of CRP and IL-6 (all p -trend > 0.05).	— Age, sex, and BMI
Scientific Reports/ Randrianasoa et al. (2016)	2016/Germany	Apparently healthy adults	220	3/73–74	Cross-sectional	Serum TMAO	8	2.83 ± 1.62	46 ± 11	41	TMAO was non significantly associated with hs-CRP level ($r = -0.01$; $p = 0.88$) a significant positive association between TMAO and TNF- α was reported ($r = 0.27$; $p = 0.001$)	Age, sex, and BMI
PLOS One/ Misalidis et al. (2016)	2016/Sweden	Patients with CKD	259	—	Cohort	Plasma TMAO	5	Control = 5.8 (3.1–13.3) Case = 53.4 (9.3–170.0)	≥18	66.79	TMAO were associated with IL-6 ($r = 0.42$; $p < 0.0001$), fibrinogen ($r = 0.43$; $p < 0.0001$) and hsCRP ($r = 0.17$; $p = 0.022$)	Age, gender, SGA, albumin, DM, and mGFR
Journal of the American Heart Association/ Meyer et al. (2016) <i>Journal of Internal Medicine/</i> Trosted et al. (2015) <i>Journal of Renal Nutrition/</i> Keyser et al. (2015) <i>Journal of the American College of Cardiology/</i> Tang et al. (2014)	2016/UK 2014/Norway 2015/USA 2014/USA	Apparently healthy adults Patients with chronic HF Adults with ESRD Patients with the history of HF	817 155 235 720	4/199–214	Cohort Cohort Cohort Cohort	Plasma TMAO Plasma TMAO Serum TMAO Plasma TMAO	10 5.2 4 2/360	2.6 (1.8–4.2) NR 43 (27.5–66) 62 ± 14	33–55 57 ± 11 55.3 66 ± 10	43	No significant difference in TMAO levels in highest versus lowest quartile ($p = 0.34$) TMAO was negatively associated with CRP level ($r = -0.08$; $p < 0.05$) TMAO concentrations inversely correlated with log CRP (-0.18 , 95% CI -0.30 to -0.06 ; $p = 0.005$)	— Age, gender, and BMI

BMI, body mass index; CAS, coronary artery syndrome; CHD, coronary heart disease; CRP, C-reactive protein; CVD, cardiovascular disease; ESRD, end stage renal disease; GFR, glomerular filtration rate; Hb-A₁C, hemoglobin A₁C; HDL, high density lipoprotein cholesterol; IL-6, interleukin; LDL, low density lipoprotein cholesterol; MetS, metabolic syndrome; SBP, systolic blood pressure; TMAO, trimethylamine N-oxide; TNF- α , tumor necrosis factor α ; T₂DM, type two diabetes mellitus; WNCA, Western Norway Coronary Angiography Cohort.

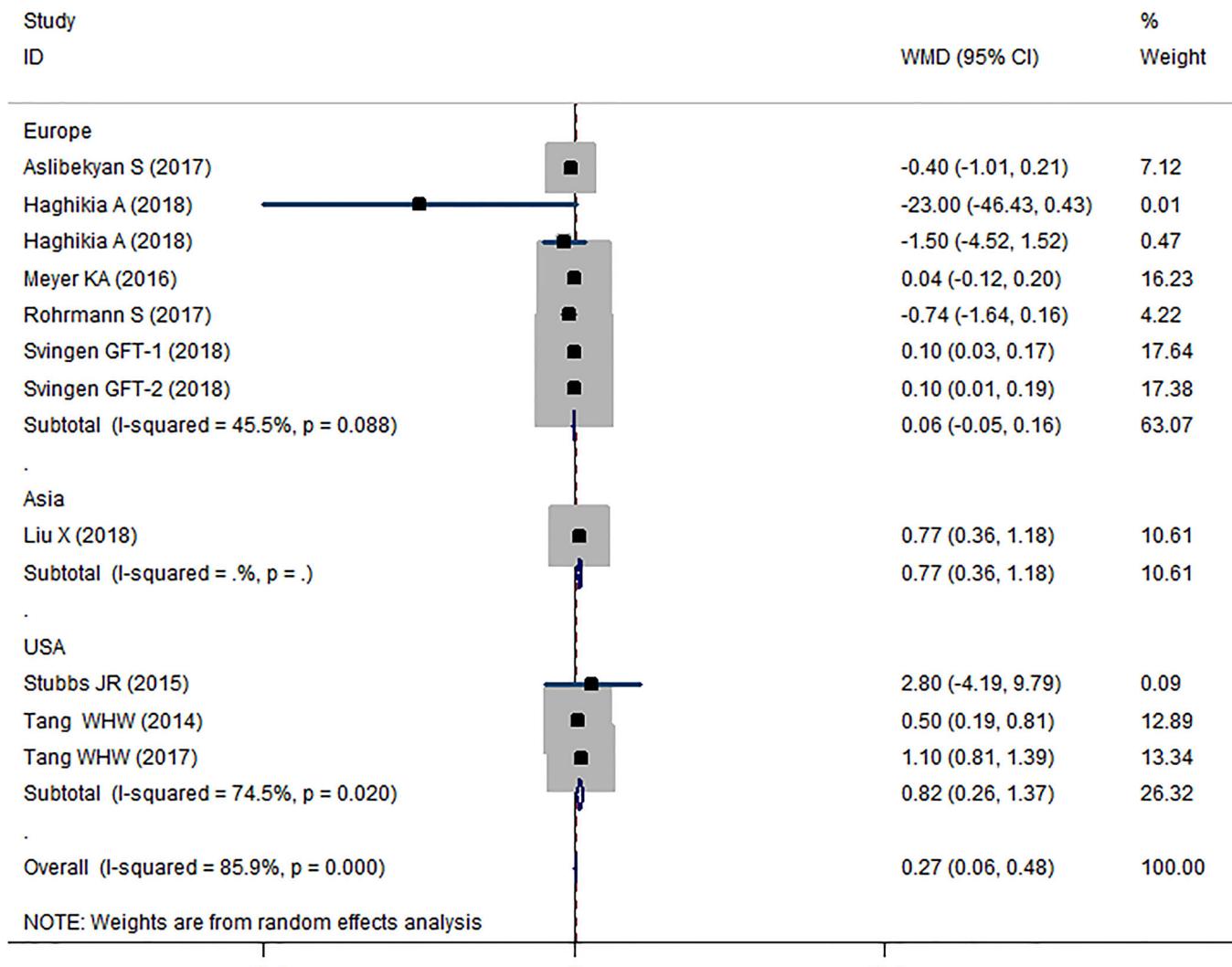


Figure 2. Weighted mean difference (WMD) of the circulating CRP concentrations in highest versus lowest trimethylamine N-oxide (TMAO) categories according to study location. I^2 represents the degree of heterogeneity.

CRD42019143010. Also, the research protocol was approved by Research Undersecretary of Tabriz University of Medical Sciences (Identifier: IR.TBZMED.VCR.REC.1399.001).

Search strategy

Electronic databases including PubMed, ProQuest, Scopus, and Embase were systematically searched up to 30 July 2019 and a total of 586 manuscripts were retrieved. No calendar date restriction was performed in our search. The reference lists of all relevant articles and reviews were also manually searched. One author (M.A.F.) performed the literature search, while another author (M.V.) reviewed uncertain cases. For any discrepancies between authors, consensus was reached through discussion between both authors. The search was restricted to only articles published in English. Although, there was no published work in other language about this subject. The search was performed according to major medical subject heading (MESH) or title abstract

according to following search strategy in PubMed: Search (“Trimethylamine N-oxide” OR “TMAO”) AND (“Fibrinogen” OR “Interleukin-17” OR “Interleukin-2” OR “Interleukin-1- β ” OR “interleukin 1” OR “Cytokines” OR “Chemokines” OR “Cytokinesis” OR “Tumor Necrosis Factor-alpha” OR “Receptors, Interleukin-6” OR “Interleukin-6” OR “hs-CRP” OR “C-Reactive Protein” OR “Anti-Inflammatory Agents, Non-Steroidal” OR “Anti-Inflammatory Agents” OR “Inflammation Mediators” OR “Inflammation”; Supplemental Table 2).

Study selection

Relevant observational studies with the design of prospective cohort, nested case-control, case-cohort, case-control or analytic cross-sectional studies were obtained and included in the current review if they: (1) reported the results according to TMAO categories for at least two categories for conducting two-class meta-analysis, (2) reported the results

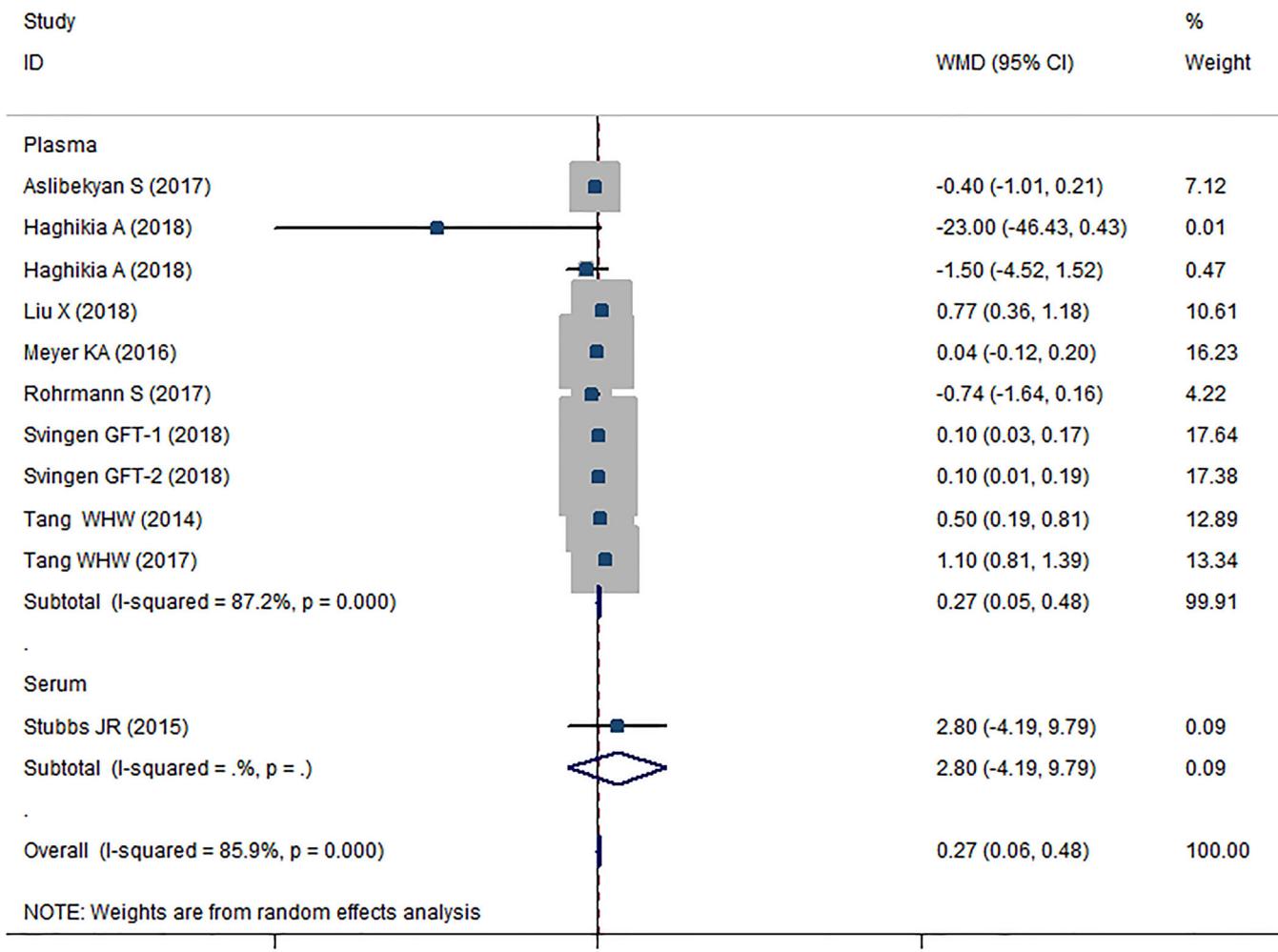


Figure 3. Weighted mean difference (WMD) of the circulating CRP concentrations in highest versus lowest trimethylamine *N*-oxide (TMAO) categories according to TMAO sample source. I^2 represents the degree of heterogeneity.

according to TMAO categories for at least three categories for conducting dose-response meta-analysis of continuous variables, (3) reported the outcome of interest as the mean and SD of continuous variables, (4) reported the number of cases and participants/person-years in each TMAO category, (5) or reported sufficient information to allow estimation of those numbers. Otherwise the study was excluded. We excluded the studies that were performed among children, pregnant or lactating women, with not relevant statistical information to be included in the meta-analysis, with interventional, experimental or in vitro designs. For cohort studies we only included the baseline characteristics while the information after follow-up was not included.

Quality assessment and data extraction

The quality assessment of the studies was performed by two independent researchers (M.A.F., M.V.) and all of the

discrepancies were resolved by discussion and involvement of the third independent researcher. For the quality assessment of the cohort and case-control studies the Newcastle Ottawa scale (NOS) (Wells et al. 2012) was used while the Agency for Healthcare Research and Quality (AHRQ) checklist was used to assess the quality of cross-sectional studies (Cho et al. 2017). The items were scored "1" if the answer was "YES," and "0" if the answer was "NO" or "UNCLEAR." The final quality assessments were as follows: low quality = 0–3; moderate quality = 4–7; high quality = 8–11. There were no quality criteria for inclusion of the studies in the current meta-analysis. The details of the studies' quality assessment are presented in Supplementary Tables 3 and 4. The data were extracted according to the standard extraction form obtaining the below information: first author's name, publication year, location of the study, design, mean age and/or age range of participants, gender, mean of range of TMAO, blood sample type for TMAO assessment (serum or plasma), blood sample type for CRP assessment (serum or plasma), number of participants/

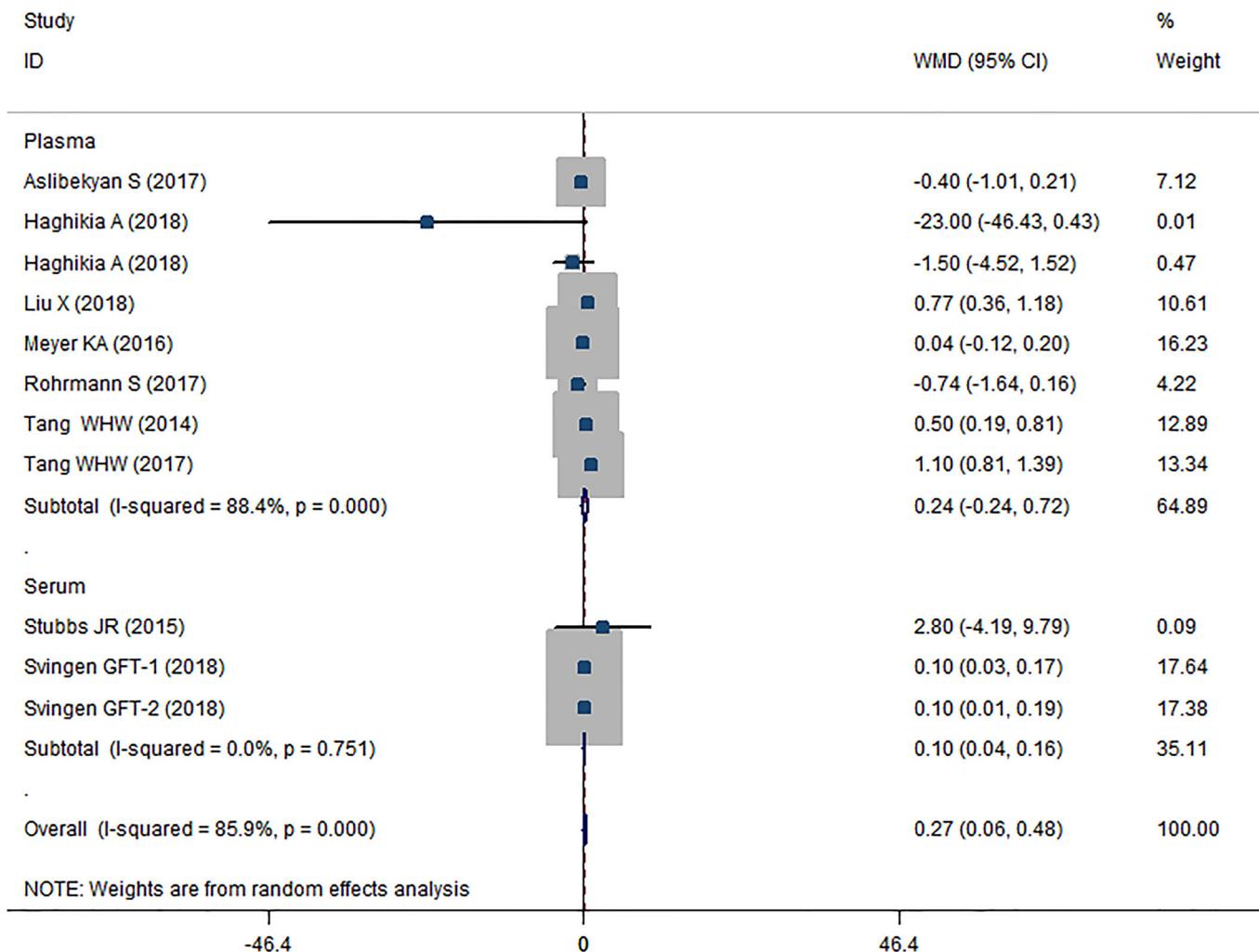


Figure 4. Weighted mean difference (WMD) of the circulating CRP concentrations in highest versus lowest trimethylamine N-oxide (TMAO) categories according to CRP sample source. I^2 represents the degree of heterogeneity.

cases in each category of TMAO, the reported mean (SD) of study outcomes across categories of TMAO. The quality scoring of the included studies are presented in Supplementary Tables 3 and 4.

Statistical analysis

The data were analyzed using STATA version 13 (STATA Corp., College Station, TX, USA), and p values less than 0.05 were considered as statistically significant.

Meta-analysis of the comparison of mean CRP in highest versus lowest TMAO categories

In two class meta-analysis, the studies who reported the mean CRP levels in highest versus lowest TMAO categories were included. The mean and SD of variable (CRP) was used to calculate the unstandardized mean differences as the effect size calculated by pooled estimate of weighted mean difference (WMD) with 95% CI in the highest versus lowest

TMAO categories as case and control groups respectively. When the mean values were missed and median and range were provided we used the method provided by Hozo, Djulbegovic, and Hozo (2005) considering the median values as best estimate of mean for sample size more than 25 and calculating SD as follows: $S^2 \approx \frac{(\frac{1}{12} \cdot (a-2m+b)^2)}{4} + (b-a)^2$.

For missing SDs, the method of Walter and Yao as improved version of “range” method was used which calculates standard deviation as $SD = (b-a)/4$ (Walter and Yao 2007; Weir et al. 2018). When the number of individuals in each category of TMAO was not provided in the manuscript, we assumed that equal number of participants are enrolled in each group. The overall effect was calculated by a Z-test, and $p < 0.05$ (2-tailed) was deemed statistically significant. Because of the high heterogeneity values (e.g., more than 50%) we applied the random effects model for the high heterogeneity values. Moreover, because the included studies would never be expected to estimate the “exact same quantities” from all the possible sources including different designs, sample sources or population

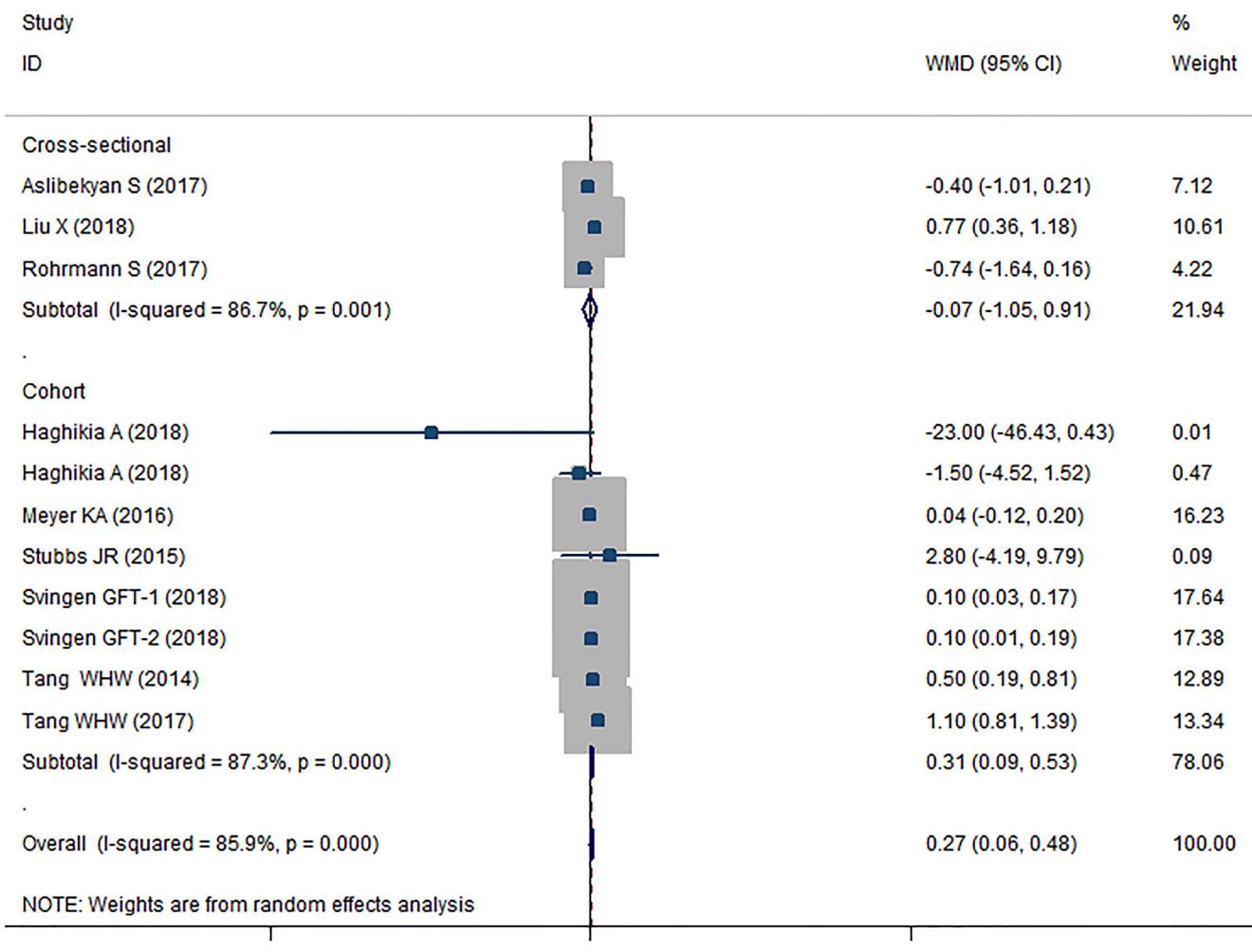


Figure 5. Weighted mean difference (WMD) of the circulating CRP concentrations in highest versus lowest trimethylamine *N*-oxide (TMAO) categories according to study design. I^2 represents the degree of heterogeneity.

sizes, therefore, the best approach to identify the effect size was the random effect model; Riley, Higgins, and Deeks (2011) suggested that using random effect model of meta-analysis with inclusion of prediction intervals by estimating the likely effect in an individual setting, will make the results to be easily applied into clinical practice. Cochran's Q test and I^2 -squared test was used to identify between-study heterogeneity classified as: $I^2 < 25\%$, no heterogeneity; $I^2 = 25\%-50\%$, moderate heterogeneity; $I^2 > 50\%$ large heterogeneity (Higgins and Thompson 2002). The heterogeneity was considered significant if either the Q statistic had $p < 0.1$ or $I^2 > 50\%$. Subgroup analysis and meta-regression analysis were performed to identify possible sources of heterogeneity according to the study design, disease status, sample size and the blood sample type for TMAO or CRP measurement, type of CRP (e.g., hs-CRP or CRP) and the prevalence of HTN, T2DM, smoking, age and gender. Begg's Funnel plots was assessed to evaluate the publication bias followed by the Egger's regression asymmetry test and Begg's adjusted rank correlation for formal statistical assessment of Funnel plot asymmetry. TMAO and CRP (or hs-CRP) values in all studies were

converted to be identical in all of measurements to $\mu\text{mol/L}$ and mg/L , respectively.

Dose response meta-analysis of the association between TMAO and CRP

For dose response meta-analysis, the eligible studies were reported the mean (SD) of continuous variable (e.g., CRP) in at least Three TMAO categories. The median point in each TMAO category was also identified. If medians were not reported in the manuscript, then approximate medians were estimated, using the midpoint of the lower and upper limits. If the highest study category was open-ended, its TMAO concentration was calculated by assuming that the interval was the same as the closest category. The lowest categories of TMAO concentration was considered as the reference dose for each study. The lowest category was considered to be the lowest dose of TMAO that had been provided for the first group. Any potential non-linear associations of CRP concentrations were performed by fractional polynominal modeling (polynomials) to explore the non-linear potential

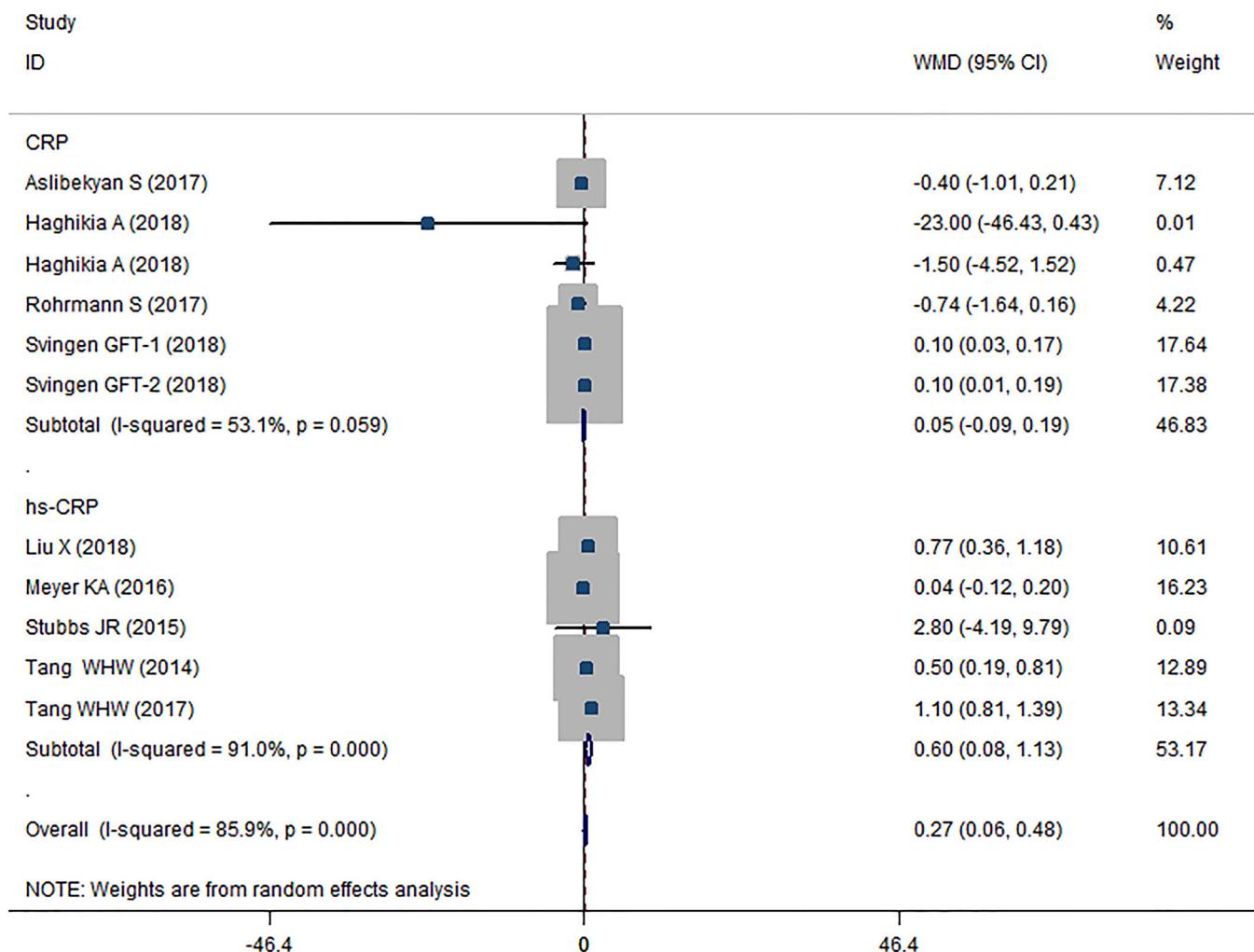


Figure 6. Weighted mean difference (WMD) of the circulating CRP concentrations in highest versus lowest trimethylamine *N*-oxide (TMAO) categories according to CRP type. I² represents the degree of heterogeneity.

effects of TMAO dosage ($\mu\text{mol/L}$) and the study-specific parameter (Fan and Gijbels 1996).

Results

Literature search, study characteristics, and the quality of included studies

Electronic databases including PubMed, ProQuest, Scopus, and Embase were systematically searched and a total of 586 manuscripts were retrieved. After removing 223 duplicates, 363 manuscripts were reviewed. No manuscripts were identified by manual search. After exclusion according to title/abstract and then full text reviews, a total of 16 papers were included in the systematic review. From these studies, eight studies were eligible to include in the meta-analysis while seven studies were eligible for dose-response meta-analysis (Meyer et al. 2016; Randrianarisoa et al. 2016; Rohrmann et al. 2016; Stubbs et al. 2016; Aslibekyan et al. 2017; Tang et al. 2017; Haghikia et al. 2018; Liu et al. 2018; Svingen et al. 2018).

et al. 2017; Svingen et al. 2018) and nine studies for two-class meta-analysis (Tang et al. 2014; Meyer et al. 2016; Rohrmann et al. 2016; Stubbs et al. 2016; Aslibekyan et al. 2017; Tang et al. 2017; Haghikia et al. 2018; Liu et al. 2018; Svingen et al. 2018). The studies by Haghikia et al (2018) and Svingen et al (2018) were included as two independent studies. The flowchart of study is presented in Figure 1 and the characteristics of the studies included in the systematic review and meta-analysis is presented in Table 1. Totally, 10 studies were cohort (Tang et al. 2014; Kaysen et al. 2015; Trøseid et al. 2015; Meyer et al. 2016; Missailidis et al. 2016; Stubbs et al. 2016; Tang et al. 2017; Haghikia et al. 2018; Svingen et al. 2018; Chou et al. 2019), 4 were cross-sectional (Randrianarisoa et al. 2016; Rohrmann et al. 2016; Aslibekyan et al. 2017; Liu et al. 2018), and 1 was nested case-control (Yu et al. 2019). The one nested-case control study was excluded from the meta-analysis. Moreover, for the included cohort studies, the baseline information were used not the information after follow-up. Therefore, all of

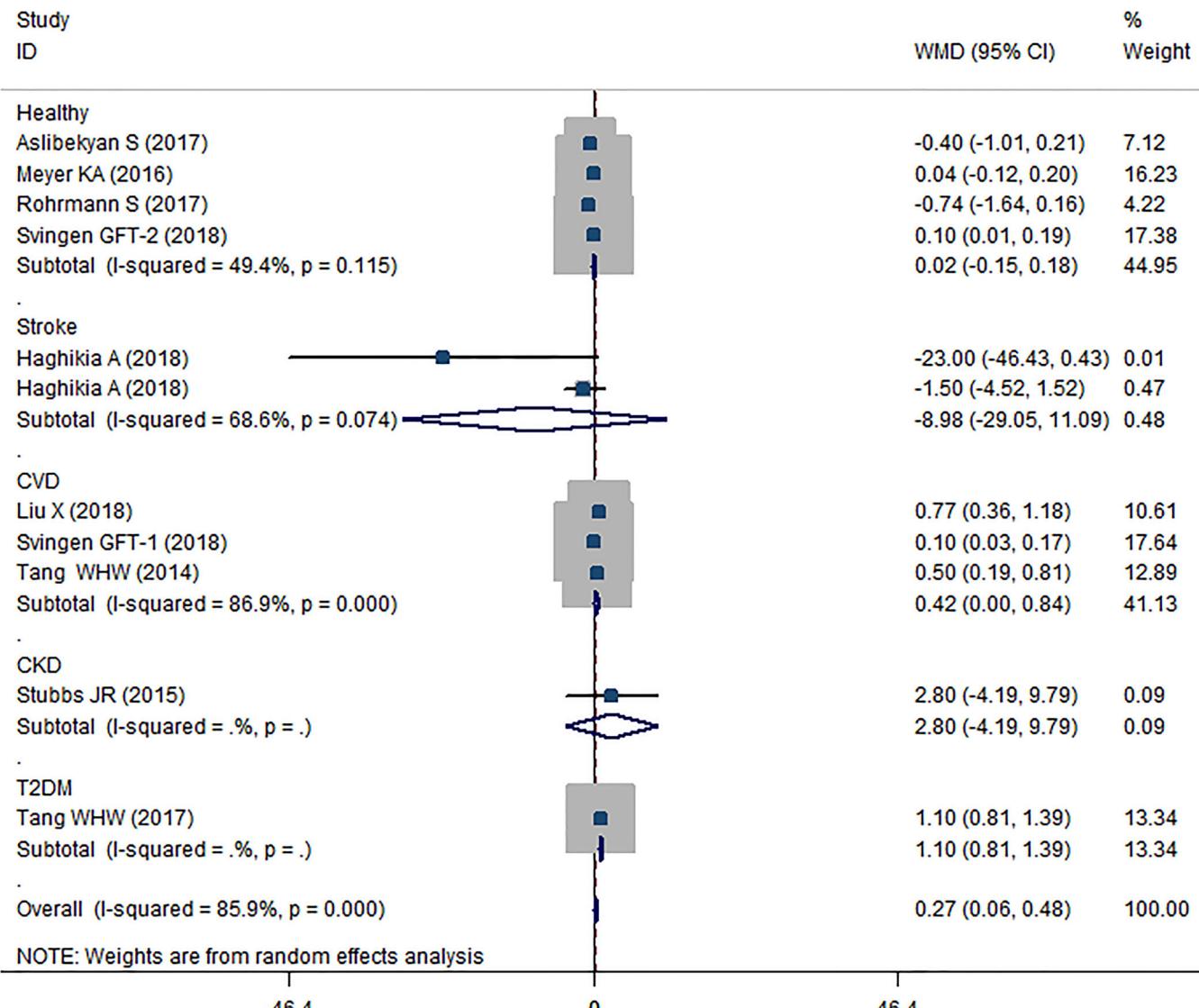


Figure 7. Weighted mean difference (WMD) of the circulating CRP concentrations in highest versus lowest trimethylamine *N*-oxide (TMAO) categories according to disease status. I^2 represents the degree of heterogeneity.

the included studies in the meta-analysis used cross-sectional data. From the inflammatory parameters, CRP has been reported in all of the 16 studies (Tang et al. 2014; Kaysen et al. 2015; Trøseid et al. 2015; Meyer et al. 2016; Missailidis et al. 2016; Randrianarisoa et al. 2016; Rohrmann et al. 2016; Stubbs et al. 2016; Aslibekyan et al. 2017; Tang et al. 2017; Haghikia et al. 2018; Lent-Schochet et al. 2018; Liu et al. 2018; Svingen et al. 2018; Chou et al. 2019; Yu et al. 2019). Totally, 12 studies found no association between CRP and circulating TMAO concentrations or no significant difference in CRP concentrations between highest versus lowest TMAO categories (Tang et al. 2014; Meyer et al. 2016; Randrianarisoa et al. 2016; Rohrmann et al. 2016; Stubbs et al. 2016; Aslibekyan et al. 2017; Tang et al. 2017; Haghikia et al. 2018; Lent-Schochet et al. 2018; Liu et al. 2018; Svingen et al. 2018; Yu et al. 2019). In the study by

Yu et al (2019), urinary TMAO was significantly associated with high serum CRP concentrations ($\beta=0.19$; $p=0.03$) while no association between TMAO and hs-CRP was reported. In the study by Svingen et al (2018), in the results of Western Norway Coronary Angiography Cohort (WECAC), CRP concentrations in the highest quartile of TMAO was higher than lowest ($p=0.03$) while no difference in the Hordaland Health Study (HUSK) cohort was reported. Three studies reported significant positive association between TMAO and CRP or a higher CRP values in the highest versus lowest TMAO categories (Missailidis et al. 2016; Svingen et al. 2018; Chou et al. 2019) and in two studies (Kaysen et al. 2015; Trøseid et al. 2015) an inverse association between TMAO and CRP was found. Other inflammatory parameters were also reported in limited number of studies including IL-6 in three studies (Missailidis

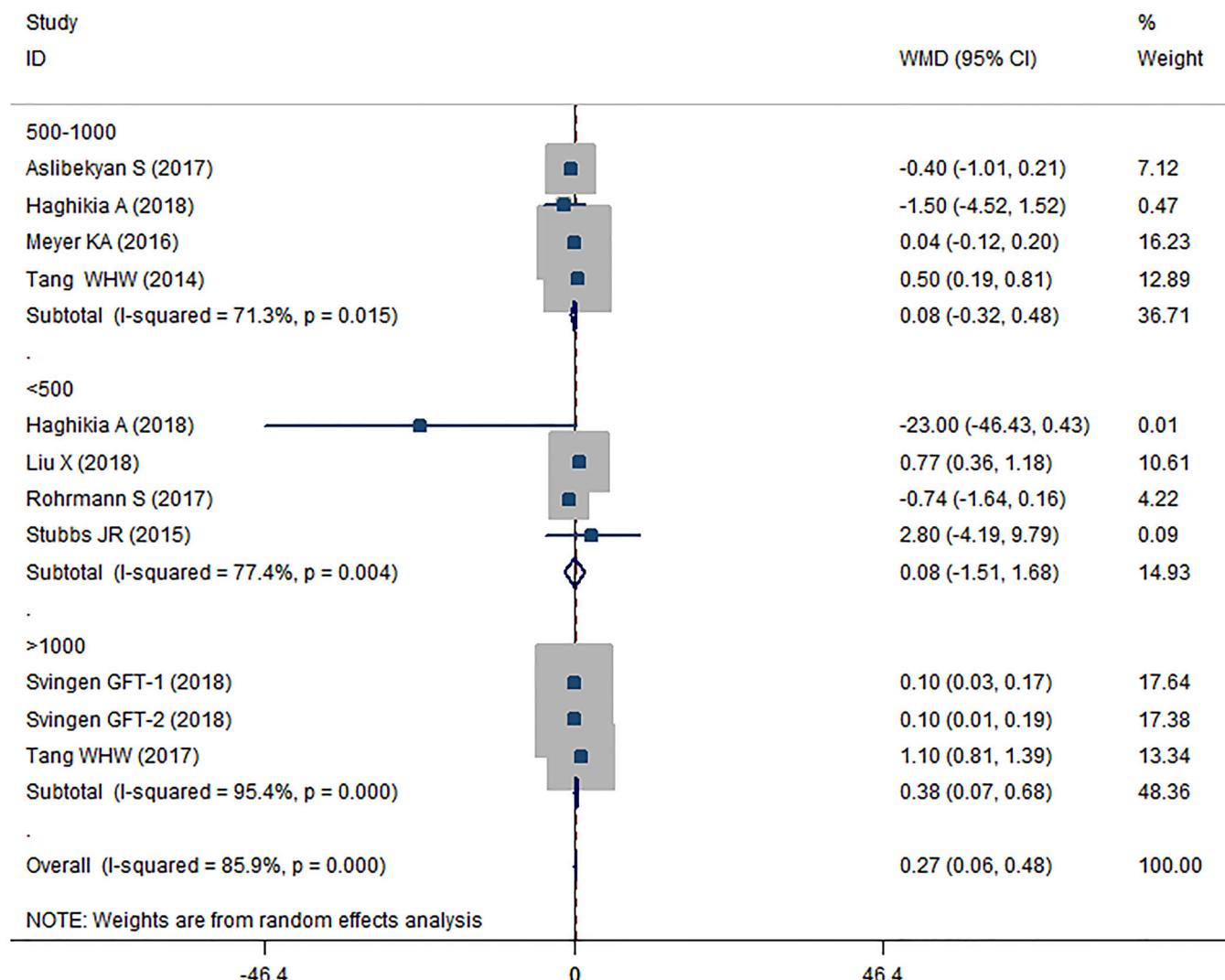


Figure 8. Weighted mean difference (WMD) of the circulating CRP concentrations in highest versus lowest trimethylamine N-oxide (TMAO) categories according to sample size of included studies. I^2 represents the degree of heterogeneity.

et al. 2016; Rohrmann et al. 2016; Lent-Schochet et al. 2018) while two studies reported positive association between TMAO and IL-6 (Missailidis et al. 2016; Lent-Schochet et al. 2018) while one other (Rohrmann et al. 2016) found no association. IL-8 was reported in one study (Lent-Schochet et al. 2018) with no significant association reported. TNF- α was also reported in three studies (Randrianarisoa et al. 2016; Rohrmann et al. 2016; Lent-Schochet et al. 2018) with positive associations in two studies (Randrianarisoa et al. 2016; Rohrmann et al. 2016) and one other found no associations (Lent-Schochet et al. 2018). The results of studies reported TNF- α , IL-6, and IL-8 were not eligible to be included in the meta-analysis. Total number of participants in the current systematic review were 13,783 individuals ranging from 50 to 4141 individuals and all of them were evaluated both genders without separate analysis. The studies were also heterogeneous in terms of geographical location while two studies were from China (Liu et al. 2018; Yu et al. 2019), one from Taiwan (Chou et al.

2019), two from Norway (Trøseid et al. 2015; Svingen et al. 2018), five from USA (Tang et al. 2014; Kaysen et al. 2015; Stubbs et al. 2016; Tang et al. 2017; Lent-Schochet et al. 2018), three from Germany (Randrianarisoa et al. 2016; Rohrmann et al. 2016; Haghikia et al. 2018), two from UK (Meyer et al. 2016; Aslibekyan et al. 2017), and one from Sweden (Missailidis et al. 2016). The studies which were included for dose-response meta-analysis (Meyer et al. 2016; Randrianarisoa et al. 2016; Rohrmann et al. 2016; Stubbs et al. 2016; Aslibekyan et al. 2017; Tang et al. 2017; Svingen et al. 2018) had 10,970 participants and the included studies in two-class meta-analysis (Tang et al. 2014; Meyer et al. 2016; Rohrmann et al. 2016; Stubbs et al. 2016; Aslibekyan et al. 2017; Tang et al. 2017; Haghikia et al. 2018; Liu et al. 2018; Svingen et al. 2018) involved a total of 12,231 participants. Eight studies analyzed plasma TMAO (Tang et al. 2014; Meyer et al. 2016; Rohrmann et al. 2016; Aslibekyan et al. 2017; Tang et al. 2017; Haghikia et al. 2018; Liu et al. 2018; Svingen et al. 2018) and in one study serum TMAO

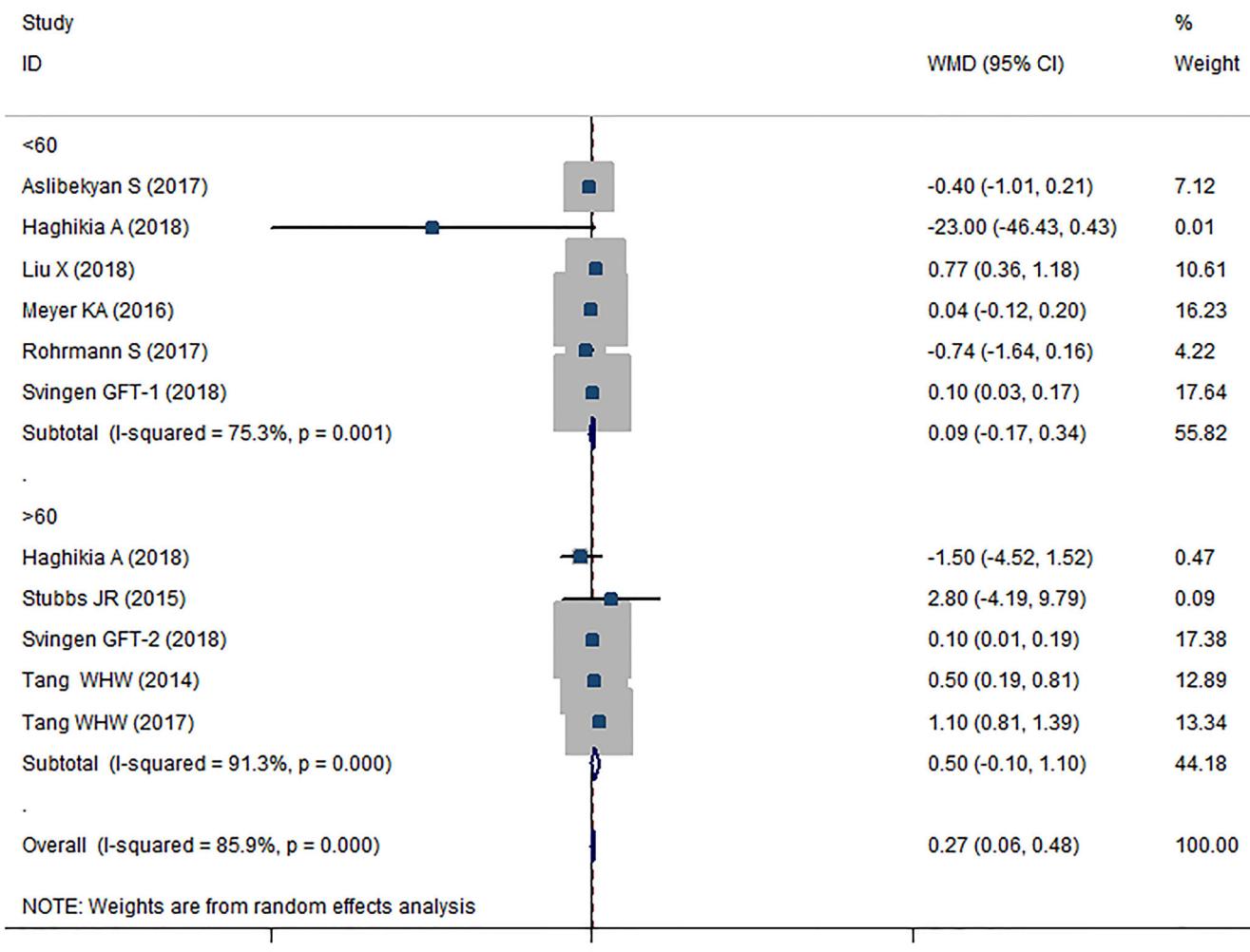


Figure 9. Weighted mean difference (WMD) of the circulating CRP concentrations in highest versus lowest trimethylamine *N*-oxide (TMAO) categories according to mean/ median age. I^2 represents the degree of heterogeneity.

(Stubbs et al. 2016) was analyzed. Two studies analyzed serum CRP while seven studies reported plasma CRP (Tang et al. 2014; Meyer et al. 2016; Rohrmann et al. 2016; Aslibekyan et al. 2017; Tang et al. 2017; Haghikia et al. 2018; Liu et al. 2018). Five studies reported hs-CRP (Tang et al. 2014; Meyer et al. 2016; Stubbs et al. 2016; Tang et al. 2017; Liu et al. 2018) while in four studies (Rohrmann et al. 2016; Aslibekyan et al. 2017; Haghikia et al. 2018; Svingen et al. 2018), CRP was reported. Most of the included cohort studies had high quality with the average NOS score of 7.63 (Tang et al. 2014; Kaysen et al. 2015; Trøseid et al. 2015; Meyer et al. 2016; Missailidis et al. 2016; Stubbs et al. 2016; Tang et al. 2017; Haghikia et al. 2018; Svingen et al. 2018; Chou et al. 2019). Accordingly, the case-control studies (Kaysen et al. 2015; Lent-Schochet et al. 2018; Yu et al. 2019) had also high quality with the average score of 6.3. Four cross-sectional studies (Randrianarisoa et al. 2016; Rohrmann et al. 2016; Aslibekyan et al. 2017; Liu et al. 2018) were also included and their scores were 7 according to AHRQ scoring checklist.

Finding from the two-class meta-analysis

The results of the two-class meta-analysis for the comparison of the CRP concentrations in highest versus lowest TMAO categories are presented in Figures 1–12. All of the eligible studies reporting the CRP concentrations in lowest and highest TMAO categories were included in two-class meta-analysis (Tang et al. 2014; Meyer et al. 2016; Rohrmann et al. 2016; Stubbs et al. 2016; Aslibekyan et al. 2017; Tang et al. 2017; Haghikia et al. 2018; Liu et al. 2018; Svingen et al. 2018). These studies were carried out in both genders and because of the high level of heterogeneity (SMD: 0.142; 95% CI: 0.094, 0.190; $p < 0.001$; $I^2 = 84.1\%$ data not shown) the random effect model was used. Accordingly, being in the highest category of TMAO was associated with 0.27 mg/L (WMD: 0.268; 95% CI: 0.058–0.479; $p = 0.013$). The subgrouping was performed according to the study location, quality, design, and sample source, the prevalence of diabetes, hypertension, age, gender and smoking status. The reported information about the medication use was not sufficient enough to perform

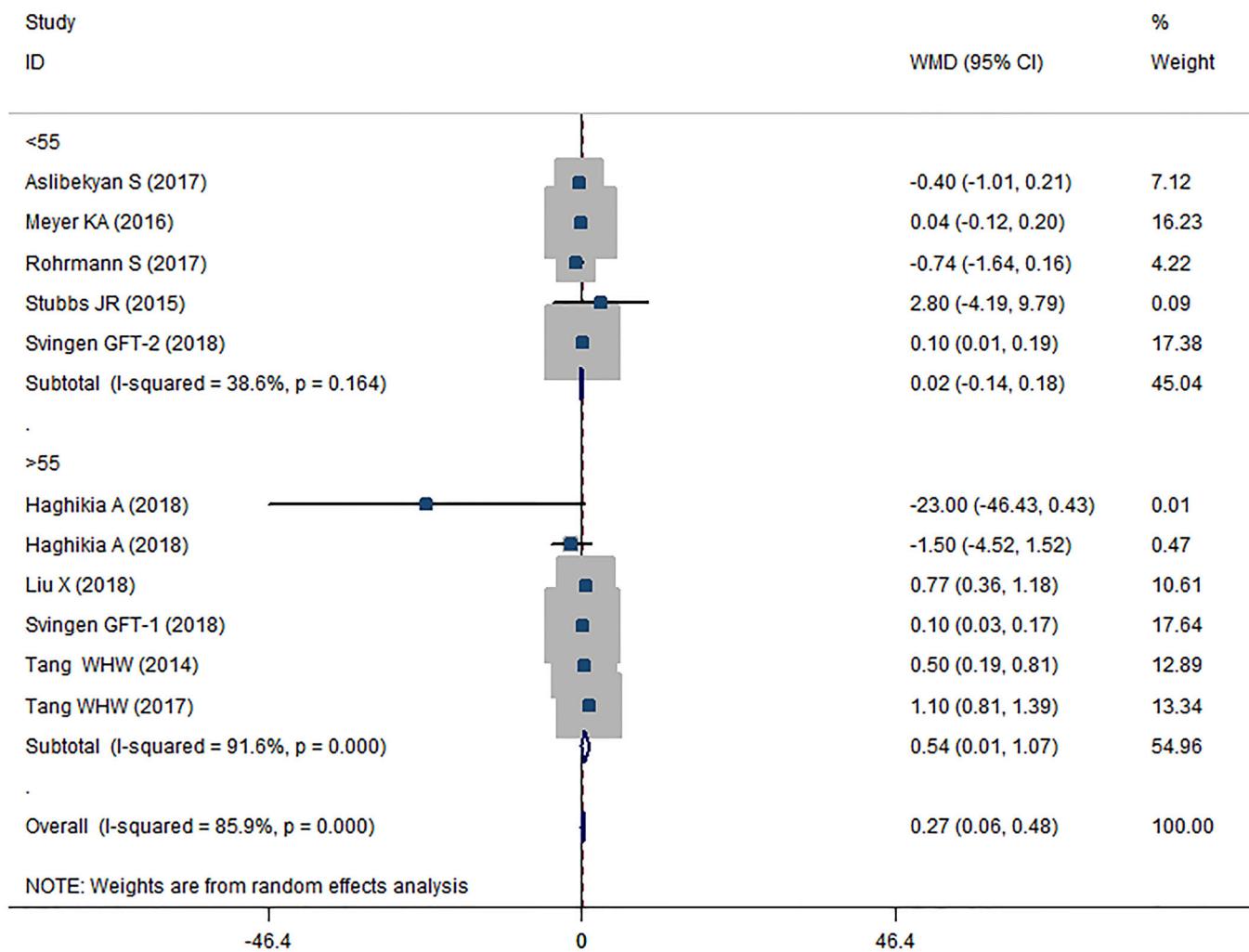


Figure 10. Weighted mean difference (WMD) of the circulating CRP concentrations in highest versus lowest trimethylamine N-oxide (TMAO) categories according to male participants percentage. I^2 represents the degree of heterogeneity.

subgrouping (Figures 2–14). The heterogeneity reduced significantly after subgrouping according to location, CRP sample source, disease status, male percent, proportion of diabetes and smoking (Table 2). Moreover, in the meta-regression analysis (Table 3), CRP type, health status and prevalence of diabetes reduced the τ^2 levels from 0.1642 to 0.147, 0.131, and 0.1023 respectively and therefore these variables could be considered as possible sources of heterogeneity.

Finding from the dose-response meta-analysis

For continuous variables which information of dose-response meta-analysis was available in adequate articles, the fractional polynomial modeling was used to evaluate the association between TMAO and mean difference in the change in the variable from the lowest category. Accordingly seven studies (Meyer et al. 2016; Randrianarisoa et al. 2016; Rohrmann et al. 2016; Stubbs et al. 2016; Aslibekyan et al.

2017; Tang et al. 2017; Svingen et al. 2018) were included for dose response association between TMAO and change in CRP concentrations; the results are presented in Figure 15 and Table 4, and there was an evidence of departure from linearity in association between increased TMAO and increased CRP (p for nonlinearity <0.05).

Publication bias

The Funnel plots are presented in Supplemental Figure 1. No evidence of publication bias was observed for the meta-analysis of the comparison of the circulating CRP in highest versus lowest TMAO categories according to Begg's and Egger's meta-bias tests (e.g., Begg test [$p = 0.788$] and Egger test [$p = 0.696$]). The Kendall score and Z-values were -11 and 0.78 respectively.

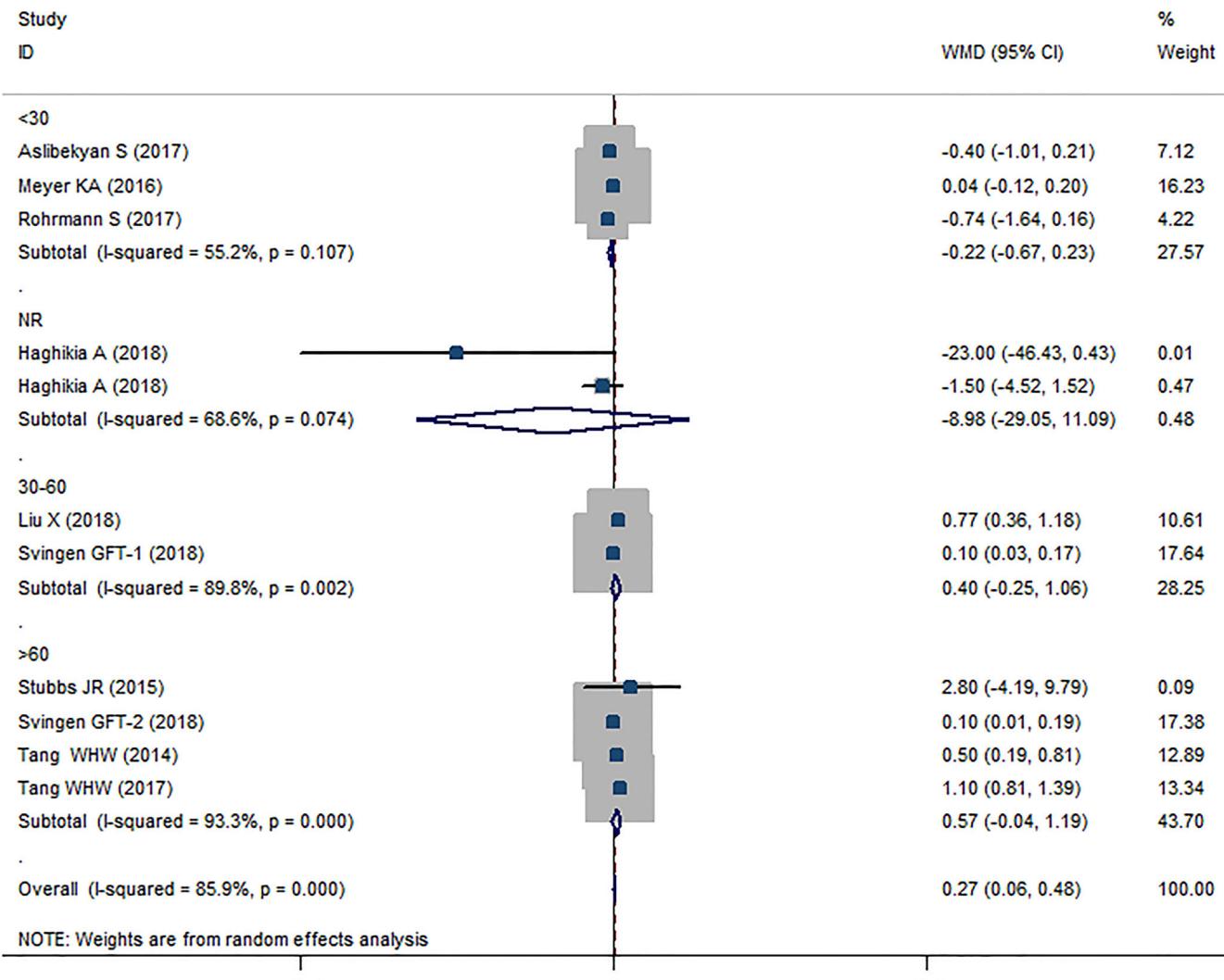


Figure 11. Weighted mean difference (WMD) of the circulating CRP concentrations in highest versus lowest trimethylamine N-oxide (TMAO) categories according to prevalence of hypertension. I^2 represents the degree of heterogeneity.

Discussion

In the current meta-analysis, for the first time, we summarized the results of the studies evaluating the association between circulating TMAO concentrations and inflammation in a large number of participants. The results demonstrated that being in the highest versus lowest TMAO categories is associated with 0.268 mg/L higher circulating CRP concentrations. There was an evidence of non-linear association between increased TMAO concentrations and increased CRP concentrations (p for nonlinearity = 0.015). Other inflammatory parameters including IL-8 and TNF- α are also reported in three studies (Missailidis et al. 2016; Rohrmann et al. 2016; Lent-Schochet et al. 2018) and because of their limited number and not-eligibility, they could not be entered into the meta-analysis. The major dietary sources of TMAO precursors are egg yolk, milk, organ and muscle meats and fish as typical foods of western diet

(Koeth et al. 2013; Hazen and Brown 2014). Although whether, circulating TMAO is affected by its dietary sources needs to be further evaluated (Rohrmann et al. 2016). The association between TMAO and inflammation has been revealed in numerous in vivo or in vitro studies (Sun et al. 2016; Chen, Zheng, et al. 2017; Chen, Zhu, et al. 2017; Yue et al. 2017; Coras et al. 2019). Several human studies reported significant positive association between TMAO and CRP or a higher CRP values in the highest versus lowest TMAO categories (Missailidis et al. 2016; Svingen et al. 2018; Chou et al. 2019). However, several other studies found no significant associations. Totally, 12 studies found no association between CRP and circulating TMAO concentrations or no significant difference in CRP concentrations between highest versus lowest TMAO categories (Tang et al. 2014; Meyer et al. 2016; Randrianarisoa et al. 2016; Rohrmann et al. 2016; Stubbs et al. 2016; Aslibekyan et al.

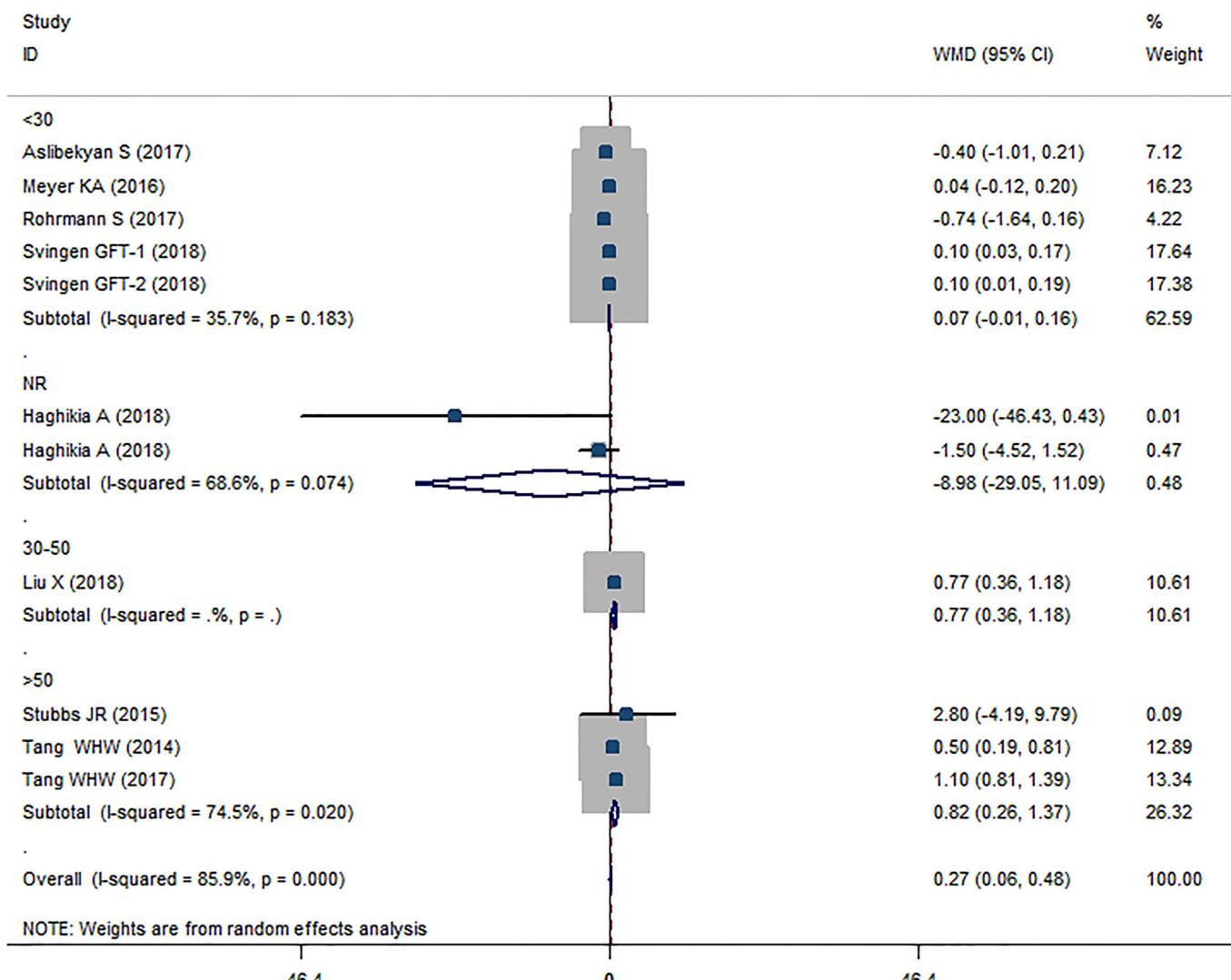


Figure 12. Weighted mean difference (WMD) of the circulating CRP concentrations in highest versus lowest trimethylamine N-oxide (TMAO) categories according to prevalence of diabetes. I^2 represents the degree of heterogeneity.

2017; Tang et al. 2017; Haghikia et al. 2018; Lent-Schochet et al. 2018; Liu et al. 2018; Svingen et al. 2018; Yu et al. 2019). It seems that the heterogeneity between studies is a major responsible factor for these inconsistencies. In our two-class meta-analysis, TMAO was in positive significant association with CRP concentrations however, there was a great source of heterogeneity and accordingly we performed a subgroup analysis to reveal the possible sources of these heterogeneities. The subgrouping revealed the role of location, CRP sample source, disease status, male percent, proportion of diabetes, and smoking possible sources of heterogeneity. However, in the meta-regression analysis only the role of health status, CRP type and prevalence of diabetes was conformed. The role of location in the heterogeneity is possibly due to the role of diet and dietary habits in regulation of circulating TMAO concentrations; diet is a powerful regulator of gut microbiota and circulating

concentrations (Claesson et al. 2012; Daniel et al. 2014). The effect of different dietary habits (e.g., Mediterranean dietary patterns, western dietary habits) on gut microbiota has been revealed (Mai et al. 2009; Claesson et al. 2012; Daniel et al. 2014) and this further explains the role of location in the heterogeneity observed in the current meta-analysis. Subgrouping also revealed reduced heterogeneity in subgroup of studies with lower than 55% of male participants compared with studies with more than 55% of males. The gender-specific association between TMAO and chronic disease has also been revealed in previous reports (Friedrich et al. 2015; Barrea et al. 2019). Diabetes, is also a determinant of TMAO concentrations since the association between circulating TMAO and increased risk of diabetes has been reported previously (Dambrova et al. 2016; Tang et al. 2017) explaining the role of diabetes as a source of heterogeneity in our study and reduced its prevalence led to a reduced

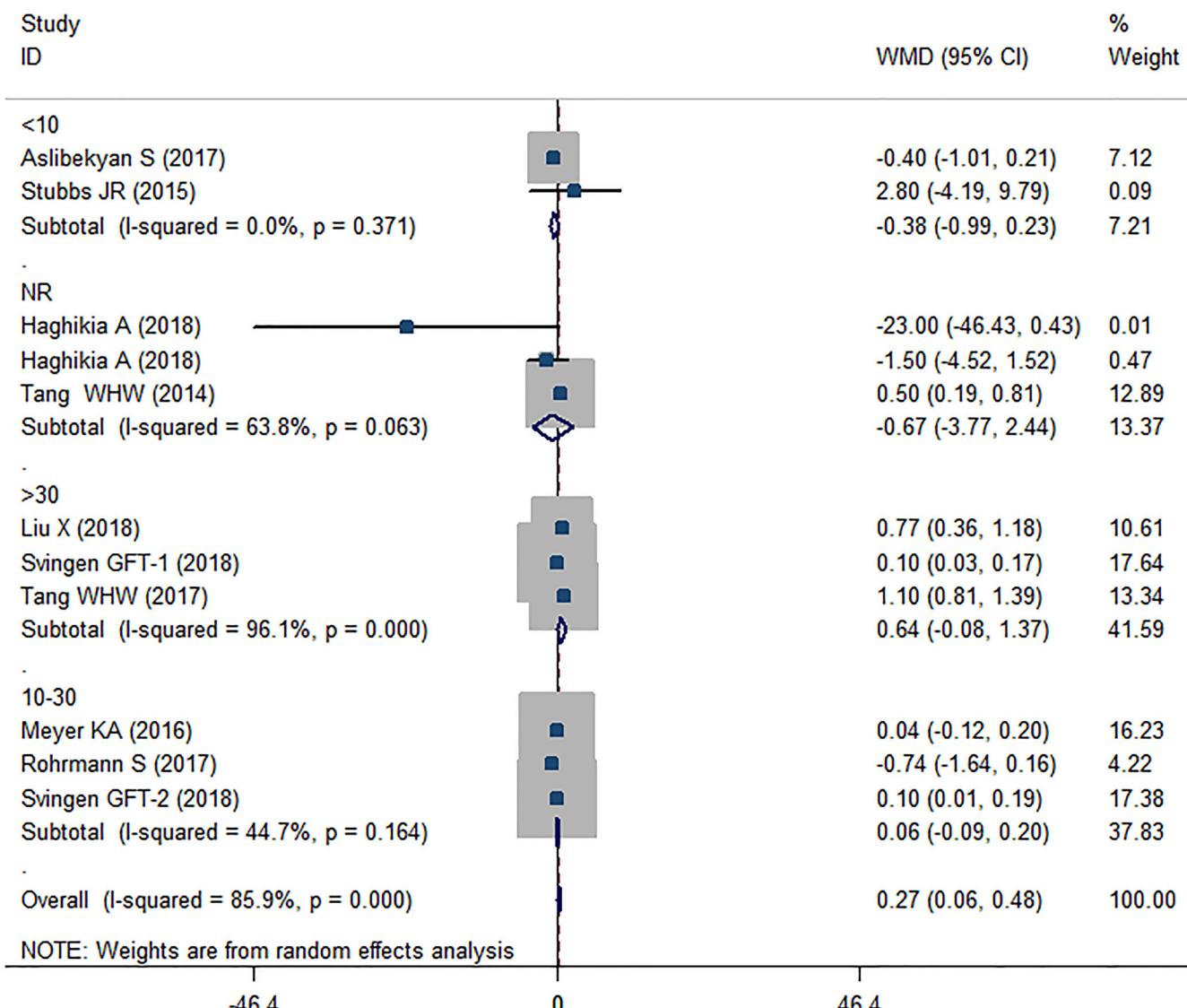


Figure 13. Weighted mean difference (WMD) of the circulating CRP concentrations in highest versus lowest trimethylamine *N*-oxide (TMAO) categories according to prevalence of current smokers. I^2 represents the degree of heterogeneity.

heterogeneity. The association between circulating TMAO and increased risk of diabetes has been revealed before (Dambrova et al. 2016; Tang et al. 2017). In the current meta-analysis performed by Zhuang et al (2019), a dose-dependent association between TMAO and risk of diabetes has been revealed. The authors suggested that TMAO-dependent elevation of *N*-nitroso compounds (NOCs), as promoters of insulin resistance, impaired glucose tolerance and obstructed hepatic insulin signal transduction might be responsible in TMAO and diabetes association. Although, further studies should be applied for clarification of underlying mechanisms of the association between TMAO and disease.

The final conclusion in the current study should be made with caution because of several reasons: first of all, because

of the observational design of included studies, causal associations could not be inferred from our findings, moreover, although, there are great between-individual variation in the conversion of choline and carnitine to TMA and then to TMAO (Miller et al. 2014), even, the gut microbiota composition affects the TMAO response to animal source foods between individuals (Cho et al. 2017) and high intake of fish and other sea-products give rise to significant increases in urinary TMAO (Zhang, Mitchell, and Smith 1999). TMAO is produced by liver oxygenation of trimethylamine (TMA), a gut bacteria-derived metabolite of choline and carnitine (Chen, Zheng, et al. 2017); accumulating evidence shows that this is not TMAO but TMA that is detrimental for the circulatory system (Jaworska, Bielinska, et al. 2019; Jaworska, Hering, et al. 2019; Jaworska, Konop, et al. 2019).

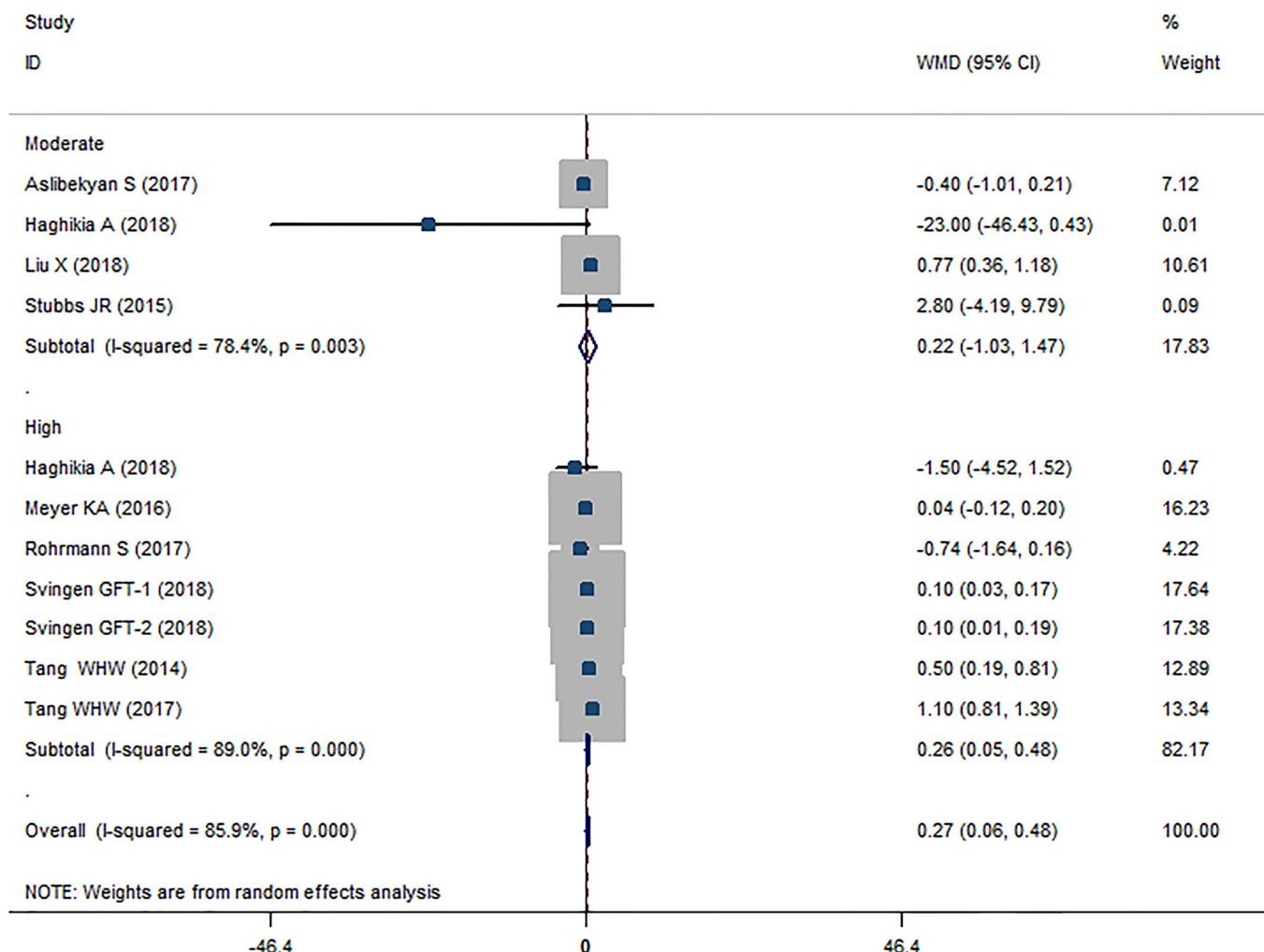


Figure 14. Weighted mean difference (WMD) of the circulating CRP concentrations in highest versus lowest trimethylamine N-oxide (TMAO) categories according to study quality. I^2 represents the degree of heterogeneity.

Although some of these studies are performed in animal models and their results could not be distributed into human studies; even, recently, there is an ongoing debate about whether TMAO is harmful or beneficial for the circulatory system (Nowiński and Ufnal 2018). Therefore, further studies are warranted to clarify the exact mechanistic pathways.

Limitations

This is the first meta-analysis evaluating the TMAO-inflammation relationship in a dose-response approach and revealed a positive association between CRP and TMAO concentrations. However, the current study has several limitations; first of all, the observational design of the included studies make us unable to infer a causal inference and therefore, the cause-effect relationship could not be elucidated; second, all of the included studies were enrolled both genders without classification and therefore, it was not possible

to study the gender-specific results, this is especially important because of the possible gender difference in circulating amounts of TMAO, its metabolism and association with CVD risk factors (Friedrich et al. 2015; Barrea et al. 2019). Although, the subgrouping according to the male gender proportion resolved this problem. Third, several potential confounders including the dietary patterns and genetic variations might affect the results. Forth, there was a high heterogeneity in the current meta-analysis with the possible identified sources of location, CRP sample source, disease status, male gender and the proportion of diabetes and smoking.

Conclusions

In the current meta-analysis we revealed the positive association between circulating TMAO and CRP both in two-class and in dose-response meta-analysis. Although numerous interventional studies are present that examined the

**Table 2.** Subgroup analysis for the prevalence of stroke in highest versus lowest TMAO categories.

Group	No. of studies (Ref.)	WMD (95% CI)	<i>P</i> within group	<i>P</i> between group *	Effect model	<i>P</i> heterogeneity	<i>I</i> ² (%)
Total*	9 (Tang et al. 2014; Meyer et al. 2016; Rohrmann et al. 2016; Stubbs et al. 2016; Aslibekyan et al. 2017; Tang et al. 2017; Haghikia et al. 2018; Liu et al. 2018; Svingen et al. 2018)	0.268 (0.058–0.479)	0.013	<0.001	Random	<0.001	85.9
Location	3 (Tang et al. 2014; Stubbs et al. 2016; Tang et al. 2017)	0.815 (0.257–1.373)	0.004	<0.001	Random	0.02	74.5
	1 (Liu et al. 2018)	0.770 (0.356–1.184)	<0.001		Fixed	—	—
Europe	5 (Meyer et al. 2016; Rohrmann et al. 2016; Aslibekyan et al. 2017; Haghikia et al. 2018; Svingen et al. 2018)	-0.017 (-0.222 to 0.188)	0.277		Random	0.088	45.5
	6 (Tang et al. 2014; Meyer et al. 2016; Rohrmann et al. 2016; Tang et al. 2017; Haghikia et al. 2018; Svingen et al. 2018)	0.262 (0.046 to 0.478)	0.017		Random	<0.001	89
Study quality	4 (Stubbs et al. 2016; Aslibekyan et al. 2017; Haghikia et al. 2018; Liu et al. 2018)	0.216 (-1.034 to 1.465)	0.73		Fixed	0.003	78.4
	3 (Tang et al. 2014; Meyer et al. 2016; Stubbs et al. 2016; Tang et al. 2017; Haghikia et al. 2018; Svingen et al. 2018)	-0.072 (-1.05 to 0.906)	0.885		Random	0.001	86.7
Design	6 (Tang et al. 2014; Meyer et al. 2016; Stubbs et al. 2016; Tang et al. 2017; Haghikia et al. 2018; Svingen et al. 2018)	0.311 (0.09 to 0.532)	0.006		Random	<0.001	87.3
	8 (Tang et al. 2014; Meyer et al. 2016; Rohrmann et al. 2016; Aslibekyan et al. 2017; Tang et al. 2017; Haghikia et al. 2018; Liu et al. 2018; Svingen et al. 2018)	0.266 (0.055 to 0.477)	0.014		Random	<0.001	87.2
TMAO sample	1 (Stubbs et al. 2016)	2.800 (-4.192 to 9.792)	0.43		Random	—	—
	7 (Tang et al. 2014; Meyer et al. 2016; Rohrmann et al. 2016; Aslibekyan et al. 2017; Tang et al. 2017; Haghikia et al. 2018; Liu et al. 2018)	0.238 (-0.241 to 0.716)	0.330		Random	<0.001	88.4
CRP sample	2 (Stubbs et al. 2016; Svingen et al. 2018)	0.100 (0.045 to 0.156)	<0.001		Fixed	0.751	0
	5 (Tang et al. 2014; Meyer et al. 2016; Stubbs et al. 2016; Tang et al. 2017; Liu et al. 2018)	0.604 (0.078 to 1.130)	0.024		Random	<0.001	91
CRP type	4 (Rohrmann et al. 2016; Aslibekyan et al. 2017; Haghikia et al. 2018; Svingen et al. 2018)	0.052 (-0.089 to 0.193)	0.468		Random	0.051	53.1
	4 (Meyer et al. 2016; Rohrmann et al. 2016; Aslibekyan et al. 2017; Svingen et al. 2018)	0.016 (-0.152 to 0.183)	0.854	<0.001	Random	0.115	49.4
Disease status	3 (Tang et al. 2014; Liu et al. 2018; Svingen et al. 2018)	0.420 (0.004 to 0.836)	0.048		Random	<0.001	86.9
	1 (Haghikia et al. 2018)	-8.982 (-29.05 to 11.09)	0.38		Random	0.074	68.6
CKD	1 (Stubbs et al. 2016)	2.800 (-4.192 to 9.792)	0.433		Fixed	—	—
	1 (Tang et al. 2017)	<0.001			Fixed	—	—

Sample size		1.10 (0.807 to 1.393)	0.149	Random	0.004	77.4
<500	4 (Rohrmann et al. 2016; Stubbs et al. 2016; Haghikia et al. 2018; Liu et al. 2018)	0.083 (-1.514 to 1.68)	0.919	Random	0.015	71.3
500–1000	4 (Tang et al. 2014; Meyer et al. 2016; Aslibekyan et al. 2017; Haghikia et al. 2018)	0.079 (-0.323 to 0.481)	0.78	Random	<0.001	95.4
≥1000	2 (Tang et al. 2017; Svartengen et al. 2018)	0.376 (0.073 to 0.678)	0.015	Random		
Mean/median age (y)		<0.001				
<60	6 (Meyer et al. 2016; Rohrmann et al. 2016; Aslibekyan et al. 2017; Haghikia et al. 2018; Liu et al. 2018; Svartengen et al. 2018)	0.087 (-0.168 to 0.343)	0.503	Random	0.001	75.3
≥66	4 (Tang et al. 2014; Stubbs et al. 2016; Tang et al. 2017; Haghikia et al. 2018; Svartengen et al. 2018)	0.499 (-0.105 to 1.103)	0.015	Random	<0.001	91.3
Male (%)						
<55	5 (Meyer et al. 2016; Rohrmann et al. 2016; Stubbs et al. 2016; Aslibekyan et al. 2017; Svartengen et al. 2018)	0.023 (-0.136 to 0.182)	0.779	0.033	0.164	38.6
≥55	5 (Tang et al. 2014; Tang et al. 2017; Haghikia et al. 2018; Liu et al. 2018; Svartengen et al. 2018)	0.535 (0.005 to 1.065)	0.048		<0.001	91.6
HTN (%)						
<30	3 (Meyer et al. 2016; Rohrmann et al. 2016; Aslibekyan et al. 2017)	-0.22 (-0.667 to 0.228)	0.336	<0.001	Random	0.107
30–60	2 (Liu et al. 2018; Svartengen et al. 2018)	0.403 (-0.251 to 1.056)	0.227		Random	0.002
≥60	3 (Tang et al. 2014; Stubbs et al. 2016; Tang et al. 2017; Svartengen et al. 2018)	0.573 (-0.042 to 1.188)	0.068		Random	<0.001
NR	1 (Haghikia et al. 2018)	-8.982 (-29.05 to 11.09)	0.380		Random	0.074
Diabetes (%)						
<30	4 (Meyer et al. 2016; Rohrmann et al. 2016; Aslibekyan et al. 2017; Svartengen et al. 2018)	0.073 (-0.009 to 0.156)	0.081		Random	0.183
30–50	2 (Tang et al. 2014; Liu et al. 2018)	0.599 (0.344 to 0.855)	<0.001	Fixed	0	0
≥50	2 (Stubbs et al. 2016; Tang et al. 2017)	1.103 (0.81 to 1.396)	<0.001		Random	0.120
NR	1 (Haghikia et al. 2018)	-8.982 (-29.05 to 11.09)	0.38		Random	0.074
Current smoking (%)						
<10	2 (Stubbs et al. 2016; Aslibekyan et al. 2017)	-0.377 (-0.988 to 0.23)	0.227		Fixed	0.370
10–30	4 (Meyer et al. 2016; Rohrmann et al. 2016; Liu et al. 2018; Svartengen et al. 2018)	0.055 (-0.089 to 0.200)	0.453		Random	0.164
≥30	2 (Meyer et al. 2016; Rohrmann et al. 2016; Liu et al. 2018)	0.645 (-0.078 to 1.367)	0.080		Random	<0.001
NR	2 (Stubbs et al. 2016; Aslibekyan et al. 2017)	-0.668 (-3.77 to 2.436)	0.673		Random	0.063

CRP, c-reactive protein; HTN, hypertension; NR, not reported; T₂DM, type two diabetes mellitus; TMAO, trimethylamine N-oxide; WMD, weighted mean difference.

*The study by Haghikia et al. (2018) and Svartengen et al. (2018) had been included as two independent studies.

effects of several dietary interventions like carnitine (Zhu et al. 2014; Zhao et al. 2018), plant based-diets (Tuso, Stoll, and Li 2015; Shah et al. 2018), probiotics (Tang and Hazen 2015; Tripolt et al. 2015) on circulating TMAO concentrations, however, the results are conflicting (e.g., the higher production of TMAO after fish consumption (Cho et al. 2017)) and it will be difficult to elucidate the exact relation between diet and TMAO concentrations. Therefore, further prospective cohort or interventional studies are needed to better elucidate the role of TMAO in disease pathogenesis and also the impact of dietary habits and life style modifications in the gut microbiota and cir-

culating TMAO concentrations.

Abbreviations

AHRQ	Agency for Healthcare Research and Quality
CI	confidence interval
CRP	C-reactive protein
NOS	Newcastle Ottawa Scale
PROSPERO	International Prospective Register of Systematic Reviews
TMAO	trimethylamine oxide
TNF- α	tumor necrosis factor α
WMD	weighted mean difference

Disclosure statement

The authors declare that there is no conflict of interest.

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Table 3. Meta regression analysis for the possible sources of heterogeneity.

CRP	τ^2	p	95% CI
Estimate of between-study variance	0.1642		
By region (USA versus others)	0.121	0.065	−0.0576 to 1.593
By study quality (high versus others)	0.294	0.959	−1.288 to 1.228
By design (cohort versus others)	0.270	0.499	−0.757 to 1.442
By CRP type (hs-CRP versus CRP)	0.147	0.049	0.004 to 1.479
By health status (healthy versus others)	0.131	0.050	−0.435 to 0.0516
By sample size (≥ 1000 versus others)	0.279	0.467	−0.685 to 1.378
By CRP sample type (plasma or serum)	0.288	0.830	−1.055 to 1.283
By TMAO sample type (plasma or serum)	0.2433	0.559	−12.22 to 7.052
By age (≥ 60 versus others)	0.215	0.273	−0.4530 to 1.421
By male% (≥ 55 versus others)	0.129	0.073	−0.0761 to 1.426
By HTN % (≥ 60 versus others)	0.216	0.188	−0.3385 to 1.488
By diabetes% (≥ 50 versus others)	0.1023	0.047	0.0258 to 2.014
By smoking (≥ 30 versus others)	0.1758	0.109	−0.178 to 1.49

CI, confidence interval; CRP, C-reactive protein; HTN, hypertension; TMAO, trimethylamine N-oxide.

The bold values represent the significant threshold ($P < 0.05$).

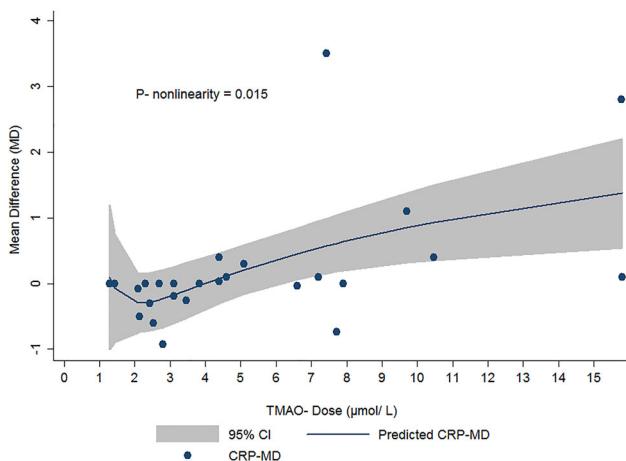


Figure 15. Dose–response association between the circulating trimethylamine N-oxide (TMAO) and CRP concentrations. Linear relation (solid line) and 95% CI (gray area) of mean difference in CRP concentrations by 1 $\mu\text{mol/L}$ increment of circulating TMAO.

Table 4. Non-linear association between TMAO and CRP.

CRP	Mean difference	Coefficient	Standard error	t	$p > t $	95% CI
Dose_1		−1.55	0.61	−2.57	0.018	−2.81 to 0.29
Dose_2		−0.610	0.27	−2.28	0.015	−1.16 to −0.055
_cons		0.28	0.21	1.37	0.186	−0.15 to 0.71

CI, confidence interval; CRP, C-reactive protein; TMAO, trimethylamine N-oxide.

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