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Effects of a diet based on the Dietary Guidelines on vascular health and TMAO in women with cardiometabolic risk factors

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Abstract

Background and aims: Recent evidence links trimethylamine oxide (TMAO) to endothelial dysfunction, an early indicator of cardiovascular disease. We aimed to determine whether short-term consumption of a diet patterned after the 2010 Dietary Guidelines for Americans (DGA) would affect endothelial function, plasma TMAO concentrations, and cardiovascular disease risk, differently than a typical American Diet (TAD).

Methods and results: An 8-wk controlled feeding trial was conducted in overweight/obese women pre-screened for insulin resistance and/or dyslipidemia. Women were randomized to a DGA or TAD group (n = 22/group). At wk0 (pre-intervention) and wk8 (post-intervention) vascular age was calculated; endothelial function (reactive hyperemia index (RHI)) and augmentation index (AI@75) were measured using EndoPAT, and plasma TMAO was measured by LC-MS/MS. Vascular age was reduced in DGA at wk8 compared to wk0 but TAD wk8 was not different from wk0 (DGA wk0: 54.2 ± 4.0 vs. wk8: 50.5 ±3.1 (p = 0.05), vs. TAD wk8: 47.7 ± 2.3). Plasma TMAO concentrations, RHI, and AI@75 were not different between groups or weeks.

Conclusion: Consumption of a diet based on the 2010 Dietary Guidelines for Americans for 8 weeks did not improve endothelial function or reduce plasma TMAO.

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Author contributions

NLK, SHA and JWN designed the controlled feeding trial; SK and NLK conducted the controlled feeding trial and generated clinical data, JWN, TLP and ERG developed technical analytical assays measuring TMAO and related metabolites, ERG measured all TMAO related metabolites, SK conducted statistical analyses, SK, BJB, NLK and SHA wrote the manuscript, all authors read and approved the final version of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.numecd.2021.09.013.

Clinicaltrials.gov: NCT02298725.

Keywords

Dietary guidelines diet pattern; Endothelial dysfunction; Controlled feeding trial

1. Introduction

Recent evidence suggests that plasma TMAO concentrations are elevated in kidney disease [1] cardiovascular disease [2,3], and even exhibit a dose-dependent relationship with severity of atherosclerotic disease [4]. But this has not been established in all populations [5] and is still being investigated [6]. Understanding the underlying mechanism between TMAO and CVD is ongoing, may involve HDL [7], inflammation [8], or endothelial dysfunction [9,10], however, whether any of these are causal or simply correlated remains unclear [11].

Several studies thus far have focused on mechanistic links between specific dietary nutrients and TMAO, since TMAO is produced from dietary choline and carnitine. Red meat is an enriched source of choline and carnitine, as are eggs [12]; other foods–cereals, fruits, and vegetables–contain little, if any, carnitine, and are not as rich a source of choline as meat, fish, or eggs [13,14]. In the US, red meat is a primary source of carnitine [13,15]. There is some debate about the association between choline and cardiovascular disease risk [16]. While this putative link could be via TMAO or homocysteine concentrations [17], TMAO remains the strongest contender [18]. Bacterial metabolism of dietary choline and carnitine in the small intestine produces the metabolic intermediate trimethylamine (TMA) [19,20]. TMA is absorbed and subsequently oxidized by one or more hepatic flavin-containing mono-oxygenases (FMO) to generate TMAO [19,21,22].

Evidence linking dietary patterns (including animal and vegetable protein sources), TMAO, and cardiovascular disease are sparse and inconclusive. Studies comparing vegetarians to omnivores demonstrated that vegetarians have lower TMAO concentrations [19] and reduced cardiovascular disease risk [23]. However, cause-effect cannot be ascertained by these associations. Recently, a 10-y follow-up cohort (n = 760) from the Nurses' Health Study reported a positive association between change (10 y minus baseline) in circulating TMAO and change in CHD risk [24]. Further, this association was made stronger by poor diet quality, as assessed by the Alternate Healthy Eating Index score [25] (a modified version of the Healthy Eating Index (HEI) score focused on foods and nutrients set forth by the Dietary Guidelines for Americans (DGA)). However, the CARDIA study did not identify any relationship between TMAO and atherosclerosis [26]. To further add complexity, while some foods (red meat, eggs) have been shown to increase TMAO concentrations, and to be positively associated with cardiovascular disease, fish has been shown to increase TMAO concentrations [27] but is often included as a dietary recommendation to protect against cardiovascular disease. Thus, the underlying associations between diet and TMAO remain unclear.

While several nutrition studies focus on biochemical aspects of single nutrients, as mentioned earlier, most policy recommendations are made at a diet pattern level, the most relevant to the US being the DGA. The DGAs are indicated for use by the public, with the

goal of helping people reduce chronic disease risk and promoting health [28]. It is therefore pertinent to determine if following the DGA pattern is capable of improving vascular health, or reducing circulating TMAO concentrations and its precursor metabolites, as this influence cardiovascular disease risk.

We recently completed the first randomized controlled feeding trial comparing a diet based on the DGA (2010) to that of a typical American Diet (TAD) [29]. The objective of this secondary analysis was to determine whether a diet based on the USDA's 2010 DGA, differentially affects plasma TMAO concentrations and endothelial function using peripheral arterial tonography, compared to a typical American Diet (TAD). We hypothesized that an 8wk-DGA intervention, which has lower red meat allowance combined with overall higher nutrient-density and quality, would improve vascular function and reduce circulating TMAO, compared to TAD.

2. Materials and methods

2.1. Study population

A randomized (1:1 block randomization, blocks of 2), double-blind, controlled 8-wk intervention was conducted in overweight and obese women (BMI between 25.1 and 39.9 kg/m^2) aged 20–65 y, with one or more characteristics of metabolic syndrome, defined as high fasting plasma glucose (>100 mg/dL but <126 mg/dL or 2 h postprandial oral glucose tolerance test (OGTT) > 140 mg/dL but <199 mg/dL) or fasting triglycerides (>150 mg/dL) or low (<50 mg/dL) HDL-cholesterol (HDL-c). We chose to study women because not only did the prevalence of being overweight and obese increased in women more than men between the years of 1988–2006, but also the prevalence of metabolic syndrome increased in women more than men (by 6%) [30]. Since metabolic syndrome is a precursor to chronic metabolic diseases such as type 2 diabetes and cardiovascular disease [31], these criteria were chosen. Screened and recruited women were randomly assigned to the DGA or TAD group (n = 22 DGA and 22 TAD). The study is registered at clinicaltrials.gov (identifier: NCT02298725) and was approved by the Institutional Review Board at University of California, Davis (UC Davis).

2.2. Diet intervention

Detailed information about the design and implementation of this dietary intervention has been previously published [32]. Two 8-d cyclic menus were developed, one for DGA and one for TAD, designed to maintain energy balance in participants. The DGA diet was designed based on the DGA 2010 [33], while the TAD diet was based on what was reported by women between the ages of 20–65 y in the "What We Eat in America Survey" from National Health and Nutrition Examination Survey (NHANES intake data 2009–2010). All food and beverages meant for consumption were prepared, packaged, and provided by the Metabolic Kitchen and Human Feeding Laboratory at the Western Human Nutrition Research Center (WHNRC). The diets were blinded to participants by using same foods and dishes in both diets, but essentially varying in proportions to meet dietary patterns. The menu had a total of 78 recipes, of which 16 had shared ingredients that matched the proportions for DGA and TAD diets respectively, but was assembled in ways to avoid bias

[32]. For instance, foods such as hot dogs (most likely associated with a TAD) and fish (more likely to be associated with a DGA) were blended into recipes, instead of being presented as such, to avoid any visual bias. Another example was to layer the ingredients that went into making pizza to show more cheese for the DGA and more vegetables on the TAD, to avoid implicit biases that might suggest to volunteers the type of diet they are on. Aside from the study dietitian and key kitchen personnel, study investigators were also blinded to treatment assignment. And participants were not told what diet they were on and did not meet other study participants (on test days or while eating at the center on specific days) to compare menu plans. Food and drinks were picked up twice a week by participants, with detailed instructions for their storage, re-heating/preparation, and consumption. Diets were developed to maintain energy balance, so as to evaluate the effect of the pattern alone. Dietary adherence was evaluated using checklists that participants filled out for each day they were in the study. In addition, participants were asked to return empty containers just as it is (without emptying out what's leftover after eating, no washing or cleaning), biweekly, which were weighed back by kitchen staff. Participants' body weights were also measured during these visits. The weigh-backs from returned containers were used to quantify adherence to consuming provided food and drinks. Based on weight maintenance and returned unconsumed foods, adherence to the diet was estimated at ~95%. The primary red meat sources in both DGA and TAD diets were beef, cold cuts, and sausage. DGA diet had an average of 26 ± 1 g/d of red meat, while TAD had an average of 88 ± 4 g/d of red meat, the primary source of carnitine. Prominent sources of dietary choline included red meat, fish, and seafood (1.1oz/d in DGA vs 0.6 oz/d in TAD), dairy (3.3 cup equivalents in DGA vs 1.5 cup equivalents in TAD), and eggs (~0.45 oz/d in both diets).

2.3. Testing paradigm

Plasma samples were collected following an overnight fast at baseline (wk0), and during the 2nd (wk2) and 8th (wk8) weeks of dietary intervention. Figure 1 outlines the study visit schedule followed at wk0 and wk8, and wk2 had the same schedule, except the EndoPAT test. The night before study visit day all participants consumed the same pre-test dinner meal (Supplemental Table 1) to ensure uniformity in metabolites measured in overnight fasted blood. All study participants arrived at WHNRC following an overnight fast, and a fasting blood draw was obtained. This was used to measure TMAO and related metabolites as outlined below. The participants then consumed a breakfast mixed meal challenge and a standard lunch meal 360 min after the breakfast (Supplemental Table 1). Following this, the EndoPAT protocol was administered during wk0 and wk8 (outlined below).

The results of the primary outcomes (fasting and OGTT glucose and insulin levels, insulin sensitivity and resistance indices (HOMA-IR, QUICKI, Matsuda index), fasting triglycerides, LDL-c, HDL-c, and total cholesterol) have been previously published [29]. Before beginning the intervention, habitual dietary intake was assessed using Automated Self-Administered 24-h dietary assessment tool (ASA24) [34] by unannounced recalls on 2 weekdays and 1 weekend day. Data from these recalls were averaged to present habitual dietary choline and carnitine in the current report.

2.4. EndoPAT

The EndoPAT 2000 (Itamar Medical Ltd, Caesarea, Israel) was used to measure relative changes in pulse wave amplitude before and after occlusion [35]. EndoPAT technique has been validated [36] and associated with several CVD risk factors in the Framingham cohort [37]. The test was administered via placement of two probes on the index fingers of right (ischemic) and left (control) hands. After a 30 min period of resting in a supine position finger probes were fitted to a finger on each hand and baseline readings of peripheral arterial tone (PAT) were recorded for 10 min. A standard blood pressure cuff was placed on one arm (measurement arm) and inflated to achieve a pressure of about 60 mm of mercury above systolic blood pressure. This occluded blood flow in the measurement arm, which was maintained for 5 min. At the end of the occlusion period, the cuff was released, and during post-reactive hyperemia, readings of PAT were recorded for an additional 5–10 min. The Reactive Hyperemia Index (RHI) was calculated as the ratio of average pulse wave amplitude during hyperemia (60–120s of post-occlusion period) to average pulse wave amplitude during baseline in the occluded hand divided by same values in the control hand and then multiplied by a baseline correction factor. The EndoPAT device also generates Augmentation Index (AI), a measure of vascular stiffness (pulse wave reflection) that is calculated from the shape of the pulse wave recorded by the probes during baseline. AI was adjusted to a heart rate of 75 beats/min (AI@75) to correct for independent effect of heart rate on this measure [38].

2.5. Vascular age

Vascular age is calculated based on a previously published and widely used multivariable logistic regression model [39], and is mostly used as an educational tool for patients in clinical care to indicate age of their vasculature. A larger positive difference between chronological age and vascular age is indicative of greater risk of cardiovascular disease. Vascular age was calculated using the excel calculator tool for lipids available at https://framinghamheartstudy.org/fhs-risk-functions/cardiovascular-disease-10-year-risk/. Input variables required to compute these two variables included age, sex, circulating fasting concentrations of HDL-c, total cholesterol, resting systolic blood pressure, smoking status, and presence or absence of type 2 diabetes. These variables were collected as part of the study and have been reported previously, and smoking and type 2 diabetes were exclusionary criteria [29].

2.6. Dietary composite analyses of choline and carnitine

Diet composites were pooled for each day of the 8-d cyclic menu, by intervention and used in proximate analyses at Covance Laboratories (Princeton NJ). As part of this analysis, dietary choline was measured using Reineckate method [40], and carnitine was measured using an LC-MS protocol [41].

2.7. LC-MS/MS analysis of TMAO and related metabolites in plasma

The metabolites TMAO, carnitine, betaine, and choline were analyzed from fasting plasma samples using an Ultra Performance Liquid Chromatography-Mass Spectrometry/ Mass Spectrometry (UPLC-MSMS), by liquid chromatography-tandem mass spectrometry

protocol, adapted from Wang et al. [12] (See supplemental methods for details). Cystatin C was measured in plasma using a K-assay (Kamiya Biomedical Company, Seattle, WA) at wk0, 2, and 8.

2.8. Statistical analysis

Sample size calculation is provided in the primary manuscript [29]. Briefly, the study (n = 17 + 25% attrition = 22/group) was powered to detect a difference of 5.32 mIU/mL in fasting insulin between DGA and TAD groups with 80% probability and an alpha of 0.05 using a 2-tailed test. For the current manuscript, statistical analyses were performed in JMP Pro 15.1.0 (SAS Institute Cary NC) and R statistical software (version 3.6.0). Significance was determined at $p < 0.05$. All data were screened for normality using QQ plots and Shapiro Wilk tests and transformed using log, cube root, or Johnson transformation to fit normal distribution as necessary. Huber M and Cauchy distribution tests were used for outlier evaluation and none were detected. Linear mixed-effect models were used with participant as the random effect, group (DGA vs. TAD) and week (0, 2, or 8) as fixed effects, wk0 as covariate to determine if the intervention had an effect on outcome variables (i.e., TMAO; betaine; choline; creatinine; carnitine; EndoPAT-based AI@75 and RHI; and vascular age). When appropriate, Tukey's multiple comparison adjustments were used to identify differences in individual means. To add clarity, in some variables, differences between wk8 and wk0 were calculated, and differences between groups were evaluated using non-parametric van der Waerden's tests. Spearman's correlation analyses were used to identify if there were associations between outcome variables at wk0 and wk8. At wk0, all 44 participants were used in the correlational analyses, and at wk8, DGA and TAD groups were evaluated individually. For Spearman's correlation analyses, Benjamini-Hochberg (BH) multiple comparison correction was used to adjust for false discoveries.

3. Results

Anthropometric, demographic, and clinical characteristics of study participants have previously been reported [29]. Briefly, at wk0, participants were between ages of 21–64 y (49.3 ± 11.2 y, mean ± SD), BMI between 25.2 and 39.1 kg/m^2 (31.6 ± 3.8 kg/m^2, mean ± SD) and there were no differences between groups (see Supplemental Table 2). Weekly body weights are presented in Supplemental Fig. 1 (mean ± SD), weight change was −1.62 ± 1.35 kg (mean ± SD) in the DGA group, and −1.23 ± 1.52 kg (mean ± SD) in the TAD group, and was not significantly different (p = 0.49 using van der Waerden's test) and could be due to being placed on a controlled feeding regimen, not to mention well within the variability reported for bodyweight fluctuations in women [42].

3.1. TMAO and related metabolites

TMAO was not significantly different between two groups at wk0 (DGA: 4.0 ± 3.3 μM, TAD: 2.9 ± 1.44 μM, p > 0.89) or wk8 (DGA: 3.5 ±1.9 μM, TAD: 3.0 ±1.9 μM, p > 0.94). Cystatin-C concentrations were within a normal healthy range (0.63–1.3 mg/L) [43] and Cystatin-C concentration was not a significant covariate of TMAO. The TMAO precursor, choline was significantly lower in DGA group at wk8 compared to wk0 (DGA wk0: 8.1 ±1.9 μM, DGA wk8: 6.8 ±1.41 μM, p < 0.01) (mean ± SD). Plasma choline values in the TAD

group were unchanged throughout the intervention (TAD wk0: 7.2 ± 1.9 μM, TAD wk8: 7.0 ± 1.6 μM, p = 0.99) (Fig. 4). No other significant differences were identified in betaine, creatinine, or carnitine. Supplemental Table 3 provides mean, standard deviation, upper and lower confidence intervals along with the effect size (Cohen's d) comparing wk8-wk0 between DGA vs TAD.

3.2. Cardiovascular disease risk indices

We first assessed endothelial function using pulse amplitude tonometry (PAT) as described in the methods (EndoPAT). There were no significant differences in AI@75 or RHI values determined by PAT between DGA and TAD at either time point (Fig. 2). Within-group analysis failed to yield significant changes from wk0 and wk8 in AI@75 or RHI. Postprandial (360 min) triglycerides and NEFA were not significant covariates when evaluating these outcomes.

In addition to direct measures of endothelial function, we also calculated a composite measure of vascular risk. Vascular age (Fig. 3) was lower at wk8 in DGA group compared to wk0, but not TAD (DGA wk0: 54.2 ± 18.6 y, wk8: 50.6 ± 14.7 y, p = 0.05; TAD wk0: 47.1 ± 10.9 y, wk8: 47.7 ± 10.7 y, p = 1.0).

3.3. Correlation with CVD risk factors

Spearman's correlation analyses results are presented in Fig. 5. At wk0, using all subjects, AI@75 was positively associated with vascular age (rho = 0.44, BH adjusted p = 0.02) and AI@75 were positively associated with plasma carnitine (rho >0.44, BH adjusted p = 0.02 for both correlations). At wk8, in DGA group, plasma carnitine was positively associated with vascular age (rho = 0.64, BH adjusted $p < 0.05$). Supplemental Fig. 2 shows results from correlational analyses done across all data, irrespective of group or week, and supports associations identified between plasma carnitine, and vascular age. There were no associations between plasma TMAO and other primary outcome parameters (fasting total cholesterol, LDL or HDL cholesterol, glucose or insulin) at wk0, wk2, or wk8. Correlations between choline, creatinine, and carnitine with primary outcome variables did not withstand when using multiple comparison corrections.

3.4. Habitual dietary intake (ASA24) and intervention diet choline and carnitine (from composite analysis)

Before the intervention, typical near-term habitual dietary intakes of choline, based on self-reported ASA24 recalls was estimated at 333.5 ± 98.6 mg/d (mean ± SEM) by women in DGA group, and 285.1 ± 97.4 mg/d (mean-SEM) in TAD group. During the 8wk intervention, dietary choline was lower in TAD diet group compared to DGA (mean ± SEM, DGA: 459.0 ± 45.7 mg/d; TAD: 385.3 ± 10.4 mg/d). L-carnitine was higher in TAD group compared to DGA (mean ± SEM, DGA: 26.0 ± 0.6 mg/d, TAD: 40.3 ± 0.3 mg/d).

4. Discussion

The primary objectives of the current study were to determine whether a diet based on the USDA's 2010 DGA, reduces plasma TMAO concentrations or improves peripheral vascular

function (peripheral arterial tonography) as compared to a typical American Diet (TAD). The results of our study indicate that 1) a DGA diet without weight loss does not alter plasma TMAO concentrations compared to a TAD diet, and 2) direct measures of vascular function via peripheral arterial tonography were also unchanged. Further, we investigated two composite measures of vascular risk which did indicate subtle effects. Vascular age indices were reduced for subjects consuming the DGA diet when compared to baseline, however, post-intervention values were not different between diet treatment groups. We discuss these results in detail.

In the current study, subjects consuming a DGA diet pattern did not have reduced circulating TMAO concentrations as compared to subjects consuming TAD. Since the Dietary Guidelines for Americans (DGA) suggests moderation in red meat intake [44] and red meat intake increases TMAO [45] we hypothesized that a DGA pattern would reduce plasma TMAO. Our hypothesis, that dietary red meat is associated with TMAO is supported in the literature where a single meal containing steak led to an increase in plasma TMAO [45], and in the cross-sectional KarMeN study which found significant associations of dietary beef and fish with circulating TMAO concentrations [46]. A recent randomized crossover designed to test the effects of diets containing different protein sources (red meat vs. white meat vs. non-meat), also demonstrated an increase in TMAO when subjects consumed a diet with red meat [12]. The data linking diet to TMAO is not entirely consistent as a cohort study in Poland failed to find an association between dietary choline or carnitine intake and circulating TMAO concentrations [47]. This suggests there is likely interindividual variability in the ability of TMAO precursors to be converted to TMAO. This variability likely is multifactorial and includes genetic (SNPs for FMOs, albeit mildly impactful [48]), gut microbiota [49], and background dietary parameters [50]. A recent study in pigs evaluated the effect of the background diet on its ability to alter TMAO production from red meat [51]. They identified that a ‘Prudent’ diet pattern, which is a nutrient-rich high-quality diet pattern when combined with red meat resulted in lower TMAO concentrations compared to a background ‘Western’ diet. Together, these results suggest that it may be necessary to evaluate a fixed amount of dietary source of TMAO (choline, carnitine, betaine), but also consider the background diet pattern to evaluate the TMAO-inducing effect of red meat.

In addition to TMAO, we also assessed several metabolites in plasma that can be converted to TMAO. Quite surprising was a reduction in plasma choline following the DGA diet pattern. This is potentially concerning as choline consumption is specifically mentioned in the 2020 DGA report as under-consumed [52] although plasma choline may not accurately reflect choline intake [53]. The recommended adequate intake for choline for adult women is 425 mg/d. Only the DGA diet reaches this recommendation, while TAD, as well as habitual intakes, fell well below this level. The food matrix could confound measures of choline and choline bioavailability can be affected by the food matrix [18]. Another possible confounder of plasma choline levels could be changes in the gut microbiome which can alter choline bioavailability [54]. However, when gut microbiota renders choline unavailable for the host, they produce more TMA, which was not seen in the current study as assessed by blood TMAO concentrations. One can only speculate that the diet pattern background, in combination with higher intake of choline altered the gut microbiota to

reduce available choline, both to the host and harmful atherogenic gut microbes. Without gut microbiome data, this is difficult to corroborate; however, future studies could factor this into their consideration while evaluating the DGA pattern. Furthermore, a combination of placing adults on a controlled diet, and regression to the mean influence the high variability observed at wk0, which then tapers and becomes narrow by wk8, so any future analyses should factor the variability of baseline microbiome while evaluating the effect of the intervention diet pattern.

There were also no differences in endothelial function or arterial stiffness between the two dietary arms. One interpretive caveat of PAT endothelial function indices (RHI and AI@75) measurements in the current report is that they were not taken in fasted state. Postprandial hypertriglyceridemia is suspected to impair endothelial function [55,56]. In fact, postprandial state lipemia-induced endothelial dysfunction is being increasingly investigated particularly after a high fat or mixed meal [57,58], and can be uniquely qualified to provide insight into metabolic disease risk [59]. In the current study, at wk0 and wk8, the meals given to the participants on the same day as PAT measurements were matched. The lack of between-day variations in acute meal compositions, and the strict timeline between ingesting the meals on the test days and PAT measurements, ensure that the effect of our interventions on the PAT parameters was studied under highly controlled conditions in the postprandial state. This enabled a direct comparison of DGA vs. TAD effects on endothelial function under the same physiological state.

TMAO may affect endothelial inflammation in mice and humans [60,61]. In the current study, however, there were no associations between endothelial function and circulating TMAO. In generally healthy women, there was no association between TMAO and endothelial function [26,62]. Further, Koay et al. [63] suggested that TMAO is associated with atherosclerotic plaque instability, which leads to downstream ischemic and myocardial perfusion issues, rather than being associated with other vascular factors such as endothelial function. Overall, our data support the notion that there is a complex link between TMAO and the long etiology of cardiovascular disease. The results also suggest that this relationship is not altered by short-term changes in diet patterns.

We reported previously [29] that the DGA intervention resulted in a moderate effect on systolic blood pressure which did not occur in the TAD group. The DGA-related reduction in vascular age is likely due to changes in blood pressure as the algorithm to calculate this includes blood pressure. The absence of any effect of the DGA diet on measured vascular and endothelial function is similar to those reported by Shah et al. [64]. Their study compared a vegan diet vs. the American Heart Association recommended diet in patients with CAD, albeit with less dietary control in a cohort of women with coronary artery disease. The endothelial function, as assessed by RHI remained unchanged, and our values are similar to those reported by Shah et al. [64]. This suggests that in women at risk for cardiometabolic disease or those with coronary artery disease, peripheral endothelial dysfunction may remain unaffected [65], and the DGA, vegan, or AHA diets do not affect otherwise ‘healthy’ parameters. Yet another study that compared a weight-reducing, plant protein-based diet against an animal protein-based diet did identify a reduction in arterial

stiffness (AI@75), but in both groups, this was a function of weight loss and not diet composition [66].

5. Strengths, limitations, and conclusions

The study was a controlled feeding trial, one of the first of its kind to evaluate the effects of a DGA based diet pattern in women at risk for cardiometabolic disease. Leveraging such a study to investigate cardiovascular risk factors such as endothelial function and TMAO and related compounds can uncover potential mechanisms that explain why diet patterns protect against or promote cardiovascular risk in the population. However, the sample size was small, and future studies should plan to look at whether an alteration in TMAO due to diet pattern is also mediating endothelial function as a means of altering cardiovascular disease risk. Yet, by closely monitoring compliance, focusing only on women, and with strict inclusion criteria, the study was designed to minimize technical variance and diet-independent variance. The results reported herein provide evidence suggesting that diet effects, if any, are difficult to detect in this population, at least over an 8wk period. Since we see within-group but no between-group differences, the lack of power due to small sample size is highly likely. Further, the study population was overweight/obese women with one or more cardiometabolic risk factors, and extrapolation to other population groups should be made with caution. Future evaluations are warranted in men and in younger and older persons, to understand if diet/TMAO/vascular phenotype relationships differ depending on context. Similarly, gut microbiota or genotype differences could add to inter-individual variability, which might account for the lack of response in TMAO and its related metabolites. In the current 8wk controlled feeding study, the effect of diet pattern on endothelial function or TMAO suggests no effect, and the modest improvements in the 10 y cardiovascular disease risk and vascular age with consumption of the DGA diet appear to be primarily driven by reductions in systolic blood pressure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Declaration of competing interest

BJB had funding from the National Cattlemen's Beef Association. Work on TMAO and related analyses presented here was, however, not supported by National Cattleman's Beef Association. SHA is founder and principal of XenoMed LLC, and has consulted with Abitech Ltd.; neither activity has involved work that is related to TMAO, its related molecules, or associations of these metabolites with cardiovascular function. The other authors declare no conflict of interests. The USDA is an equal opportunity employer.

References

[1]. Tang WH, Wang Z, Kennedy DJ, Wu Y, Buffa JA, Agatisa-Boyle B, et al. Gut microbiota-dependent trimethylamine N-oxide (TMAO) pathway contributes to both development of renal insufficiency and mortality risk in chronic kidney disease. Circ Res 2015;116: 448–55. [PubMed: 25599331]

[2]. Randrianarisoa E, Lehn-Stefan A, Wang X, Hoene M, Peter A, Heinzmann SS, et al. Relationship of serum trimethylamine N-oxide (TMAO) levels with early atherosclerosis in humans. Sci Rep 2016; 6:26745. [PubMed: 27228955]

[3]. Zhao Y, Wang Z. Impact of trimethylamine N-oxide (TMAO) meta-organismal pathway on cardiovascular disease. J Lab Precis Med 2020;5.

[4]. Haghikia A, Li XS, Liman TG, Bledau N, Schmidt D, Zimmermann F, et al. Gut microbiota-dependent trimethylamine N-oxide predicts risk of cardiovascular events in patients with stroke and is related to proinflammatory monocytes. Arterioscler Thromb Vasc Biol 2018;38:2225–35. [PubMed: 29976769]

[5]. Andraos S, Jones B, Lange K, Clifford SA, Thorstensen EB, Kerr JA, et al. Trimethylamine N-oxide (TMAO) is not associated with cardiometabolic phenotypes and inflammatory markers in children and adults. Curr Dev Nutr 2021;5:nzaa179.

[6]. Koay YC, Chen YC, Wali JA, Luk AWS, Li M, Doma H, et al. Plasma levels of trimethylamine-N-oxide can be increased with 'healthy' and 'unhealthy' diets and do not correlate with the extent of atherosclerosis but with plaque instability. Cardiovasc Res 2021; 117:435–49. [PubMed: 32267921]

[7]. Dong ZX, Zhang J, Luo YC, Zhao MM, Cai JG, Cheng S, et al. The correlation between trimethylamine N-oxide, lipoprotein ratios, and conventional lipid parameters in patients with unstable angina pectoris. Biosci Rep 2020:40.

[8]. Fu BC, Hullar MAJ, Randolph TW, Franke AA, Monroe KR, Cheng I, et al. Associations of plasma trimethylamine N-oxide, choline, carnitine, and betaine with inflammatory and cardiometabolic risk biomarkers and the fecal microbiome in the Multiethnic Cohort Adiposity Phenotype Study. Am J Clin Nutr 2020;111:1226–34. [PubMed: 32055828]

[9]. Sun X, Jiao X, Ma Y, Liu Y, Zhang L, He Y, et al. Trimethylamine N-oxide induces inflammation and endothelial dysfunction in human umbilical vein endothelial cells via activating ROS-TXNIP-NLRP3 inflammasome. Biochem Biophys Res Commun 2016;481:63–70. [PubMed: 27833015]

[10]. Brown AA, Hu FB. Dietary modulation of endothelial function: implications for cardiovascular disease. Am J Clin Nutr 2001;73: 673–86. [PubMed: 11273841]

[11]. Papandreou C, Moré M, Bellamine A. Trimethylamine N-oxide in relation to cardiometabolic health-cause or effect? Nutrients 2020;12.

[12]. Wang Z, Bergeron N, Levison BS, Li XS, Chiu S, Jia X, et al. Impact of chronic dietary red meat, white meat, or non-meat protein on trimethylamine N-oxide metabolism and renal excretion in healthy men and women. Eur Heart J 2019;40:583–94. [PubMed: 30535398]

[13]. Demarquoy J, Georges B, Rigault C, Royer M-C, Clairet A, Soty M, et al. Radioisotopic determination of l-carnitine content in foods commonly eaten in Western countries. Food Chem 2004;86: 137–42.

[14]. Zeisel SH, Mar MH, Howe JC, Holden JM. Concentrations of choline-containing compounds and betaine in common foods. J Nutr 2003;133:1302–7. [PubMed: 12730414]

[15]. Seline K-G, Johein H. The determination of l-carnitine in several food samples. Food Chem 2007;105:793–804.

[16]. Meyer KA, Shea JW. Dietary choline and betaine and risk of CVD: a systematic review and meta-analysis of prospective studies. Nutrients 2017;9.

[17]. Gerhard GT, Duell PB. Homocysteine and atherosclerosis. Curr Opin Lipidol 1999;10:417–28. [PubMed: 10554704]

[18]. Wiedeman AM, Barr SI, Green TJ, Xu Z, Innis SM, Kitts DD. Dietary choline intake: current state of knowledge across the life cycle. Nutrients 2018;10.

[19]. Koeth RA, Wang Z, Levison BS, Buffa JA, Org E, Sheehy BT, et al. Intestinal microbiota metabolism of l-carnitine, a nutrient in red meat, promotes atherosclerosis. Nat Med 2013;19:576–85. [PubMed: 23563705]

[20]. Tang WH, Hazen SL. The contributory role of gut microbiota in cardiovascular disease. J Clin Invest 2014;124:4204–11. [PubMed: 25271725]

[21]. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature 2011;472:57–63. [PubMed: 21475195]

[22]. Bennett BJ, Vallim TQdA, Wang Z, Shih DM, Meng Y, Gregory J, et al. Trimethylamine-N-Oxide, a metabolite associated with atherosclerosis, exhibits complex genetic and dietary regulation. Cell Metabol 2013;17:49–60.

[23]. Teixeira ReC, Molina MeC, Zandonade E, Mill JG. Cardiovascular risk in vegetarians and omnivores: a comparative study. Arq Bras Cardiol 2007;89:237–44. [PubMed: 17992380]

[24]. Heianza Y, Ma W, DiDonato JA, Sun Q, Rimm EB, Hu FB, et al. Long-term changes in gut microbial metabolite trimethylamine N-oxide and coronary heart disease risk. J Am Coll Cardiol 2020;75:763–72. [PubMed: 32081286]

[25]. Krebs-Smith SM, Pannucci TE, Subar AF, Kirkpatrick SI, Lerman JL, Tooze JA, et al. Update of the healthy eating index: HEI-2015. J Acad Nutr Diet 2018;118:1591–602. [PubMed: 30146071]

[26]. Meyer KA, Benton TZ, Bennett BJ, Jacobs DR, Lloyd-Jones DM, Gross MD, et al. Microbiota-dependent metabolite trimethylamine N-oxide and coronary artery calcium in the coronary artery risk development in young adults study (CARDIA). J Am Heart Assoc 2016;5.

[27]. Landfald B, Valeur J, Berstad A, Raa J. Microbial trimethylamine-. Microb Ecol Health Dis 2017;28:1327309. [PubMed: 28588431]

[28]. Promotion OoDPaH. 2010 dietary Guidelines. In: Agriculture UDo, services UDoHaH; 2010. p. 5–7. Washington DC.

[29]. Krishnan S, Adams SH, Allen LH, Laugero KD, Newman JW, Stephensen CB, et al. A randomized controlled-feeding trial based on the Dietary Guidelines for Americans on cardiometabolic health indexes. Am J Clin Nutr 2018;108:266–78. [PubMed: 30101333]

[30]. Rochlani Y, Pothineni NV, Mehta JL. Metabolic syndrome: does it differ between women and men? Cardiovasc Drugs Ther 2015;29: 329–38. [PubMed: 25994831]

[31]. Bentley-Lewis R, Koruda K, Seely EW. The metabolic syndrome in women. Nat Clin Pract Endocrinol Metabol 2007;3:696–704.

[32]. Krishnan S, Lee F, Burnett DJ, Kan A, Bonnel EL, Allen LH, et al. Challenges in designing and delivering diets and assessing adherence: a randomized controlled trial evaluating the 2010 dietary Guidelines for Americans. Curr Dev Nutr 2020;4:nzaa022.

[33]. UDo Agriculture. Dietary Guidelines for Americans 2010. In: Services UDoHaH, editor. Dietary Guidelines for Americans. 7th ed. ed. Washington DC: US Government Printing Office; 2010.

[34]. Subar AF, Kirkpatrick SI, Mittl B, Zimmerman TP, Thompson FE, Bingley C, et al. The Automated Self-Administered 24-hour dietary recall (ASA24): a resource for researchers, clinicians, and educators from the National Cancer Institute. J Acad Nutr Diet 2012;112: 1134–7. [PubMed: 22704899]

[35]. Axtell AL, Gomari FA, Cooke JP. Assessing endothelial vasodilator function with the Endo-PAT 2000. J Vis Exp 2010.

[36]. Moerland M, Kales AJ, Schrier L, van Dongen MG, Bradnock D, Burggraaf J. Evaluation of the EndoPAT as a tool to assess endothelial function. Int J Vasc Med 2012;2012:904141. [PubMed: 22500237]

[37]. Hamburg NM, Keyes MJ, Larson MG, Vasan RS, Schnabel R, Pryde MM, et al. Cross-sectional relations of digital vascular function to cardiovascular risk factors in the Framingham Heart Study. Circulation 2008;117:2467–74. [PubMed: 18458169]

[38]. McCrea CE, Skulas-Ray AC, Chow M, West SG. Test-retest reliability of pulse amplitude tonometry measures of vascular endothelial function: implications for clinical trial design. Vasc Med 2012;17:29–36. [PubMed: 22363016]

[39]. Groenewegen KA, den Ruijter HM, Pasterkamp G, Polak JF, Bots ML, Peters SA. Vascular age to determine cardiovascular disease risk: a systematic review of its concepts, definitions, and clinical applications. Eur J Prev Cardiol 2016;23:264–74. [PubMed: 25609227]

[40]. Glick D Concerning the reineckate method for the determination of choline. J Biol Chem 1944;156:643–52.

[41]. Starkey DE, Denison JE, Seipelt CT, Jacobs WA. Single-laboratory validation of a liquid chromatographic/tandem mass spectro-metric method for the determination of free and total carnitine in infant formula and raw ingredients. J AOAC Int 2008;91:130–42. [PubMed: 18376595]

[42]. Orsama AL, Mattila E, Ermes M, van Gils M, Wansink B, Korhonen I. Weight rhythms: weight increases during weekends and decreases during weekdays. Obes Facts 2014;7:36–47. [PubMed: 24504358]

[43]. Villa P, Jiménez M, Soriano MC, Manzanares J, Casasnovas P. Serum cystatin C concentration as a marker of acute renal dysfunction in critically ill patients. Crit Care 2005;9:R139–43. [PubMed: 15774046]

[44]. USDA. Dietary Guidelines for Americans 2015. In: Agriculture USDo, editor. 8th ed. Washington DC: US Government Printing Office; 2015.

[45]. 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.

[46]. Kruger R, Merz B, Rist MJ, Ferrario PG, Bub A, Kulling SE, et al. Associations of current diet with plasma and urine TMAO in the KarMeN study: direct and indirect contributions. Mol Nutr Food Res 2017;61.

[47]. Malinowska AM, Szwengiel A, Chmurzynska A. Dietary, anthropometric, and biochemical factors influencing plasma choline, carnitine, trimethylamine, and trimethylamine-N-oxide concentrations. Int J Food Sci Nutr 2017;68:488–95. [PubMed: 27855528]

[48]. Hartiala J, Bennett BJ, Tang WH, Wang Z, Stewart AF, Roberts R, et al. Comparative genome-wide association studies in mice and humans for trimethylamine N-oxide, a proatherogenic metabolite of choline and L-carnitine. Arterioscler Thromb Vasc Biol 2014;34: 1307–13. [PubMed: 24675659]

[49]. Brown JM, Hazen SL. The gut microbial endocrine organ: bacterially derived signals driving cardiometabolic diseases. Annu Rev Med 2015;66:343–59. [PubMed: 25587655]

[50]. Meyer KA, Bennett BJ. Diet and gut microbial function in metabolic and cardiovascular disease risk. Curr Diabetes Rep 2016;16:93.

[51]. Thøgersen R, Rasmussen MK, Sundekilde UK, Goethals SA, Van Hecke T, Vossen E, et al. Background diet influences TMAO concentrations associated with red meat intake without influencing apparent hepatic TMAO-related activity in a porcine model. Metabolites 2020;10.

[52]. Committee DGA. Scientif ic report of the 2020 dietary Guidelines advisory committee: advisory report to the secretary of agriculture and the secretary of health and human services. In: U.S. Department of agriculture ARS; 2020. p. 161. Washington, DC.

[53]. Wiedeman A, Dyer R, Innis S. Variability in plasma free choline and its relation with diet and potential plasma biomarkers. In: Experimental biology annual meeting. Boston MA: FASEB; 2015.

[54]. 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:e02481. [PubMed: 25784704]

[55]. Bae JH, Bassenge E, Kim KB, Kim YN, Kim KS, Lee HJ, et al. Postprandial hypertriglyceridemia impairs endothelial function by enhanced oxidant stress. Atherosclerosis 2001;155:517–23. [PubMed: 11254924]

[56]. Vogel RA, Corretti MC, Plotnick GD. The postprandial effect of components of the Mediterranean diet on endothelial function. J Am Coll Cardiol 2000;36:1455–60. [PubMed: 11079642]

[57]. Litwin NS, Van Ark HJ, Hartley SC, Michell KA, Vazquez AR, Fischer EK, et al. Impact of red beetroot juice on vascular endothelial function and cardiometabolic responses to a high-fat meal in middle-aged/older adults with overweight and obesity: a randomized, double-blind, placebo-controlled, crossover trial. Curr Dev Nutr 2019;3:nzz113.

[58]. Volpe GE, Wanke CA, Imai CM, Heffernan KS, Kuvin JT, Mangili A. High-fat meals do not impair postprandial endothelial function in HIV-infected and uninfected men. AIDS Res Hum Retrovir 2014; 30:881–7. [PubMed: 24892462]

[59]. Metzig AM, Schwarzenberg SJ, Fox CK, Deering MM, Nathan BM, Kelly AS. Postprandial endothelial function, inflammation, and oxidative stress in obese children and adolescents. Obesity (Silver Spring) 2011;19:1279–83. [PubMed: 21233813]

[60]. Brunt VE, Gioscia-Ryan RA, Casso AG, VanDongen NS, Ziemba BP, Sapinsley ZJ, et al. Trimethylamine-N-Oxide promotes age-related vascular oxidative stress and endothelial dysfunction in mice and healthy humans. Hypertension 2020;76:101–12. [PubMed: 32520619]

[61]. Chou RH, Chen CY, Chen IC, Huang HL, Lu YW, Kuo CS, et al. Trimethylamine N-oxide, circulating endothelial progenitor cells, and endothelial function in patients with stable Angina. Sci Rep 2019; 9:4249. [PubMed: 30862856]

[62]. Chiesa ST, Charakida M, Rapala A, Bhowruth DJ, Turner C, Dalton RN, et al. Endothelial function in healthy adults is unaffected by elevated serum trimethylamine N-oxide levels following one month of dietary choline supplementation. Circulation 2019: A10080.

[63]. Koay YC, Chen YC, Wali JA, Luk AWS, Li M, Doma H, et al. Plasma levels of TMAO can be increased with ‘healthy’ and ‘unhealthy’ diets and do not correlate with the extent of atherosclerosis but with plaque instability. Cardiovasc Res 2020.

[64]. Shah B, Newman JD, Woolf K, Ganguzza L, Guo Y, Allen N, et al. Anti-Inflammatory effects of a vegan diet versus the American heart association-recommended diet in coronary artery disease trial. J Am Heart Assoc 2018;7:e011367. [PubMed: 30571591]

[65]. Gutiérrez E, Flammer AJ, Lerman LO, Elízaga J, Lerman A, Fernández-Avilés F. Endothelial dysfunction over the course of coronary artery disease. Eur Heart J 2013;34:3175–81. [PubMed: 24014385]

[66]. Hill AM, Harris Jackson KA, Roussell MA, West SG, Kris-Etherton PM. Type and amount of dietary protein in the treatment of metabolic syndrome: a randomized controlled trial. Am J Clin Nutr 2015;102:757–70. [PubMed: 26354540]

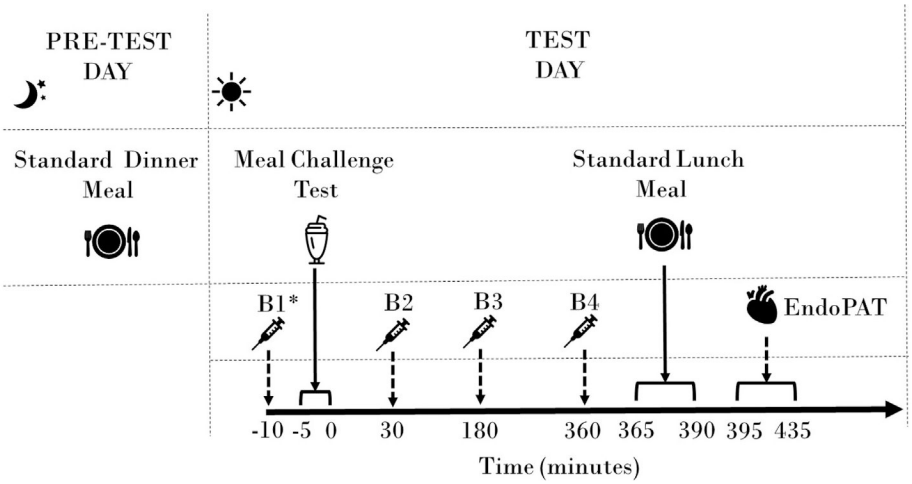

Figure 1. Testing paradigm:

The testing protocol followed for wk0 and wk8. B1 – B4 indicate blood draws at various time points in the day. Time in minutes is relative to completing the meal challenge protocol (0 min). "*" next to B1 indicates overnight fasted blood draw used to measure TMAO and related metabolites.

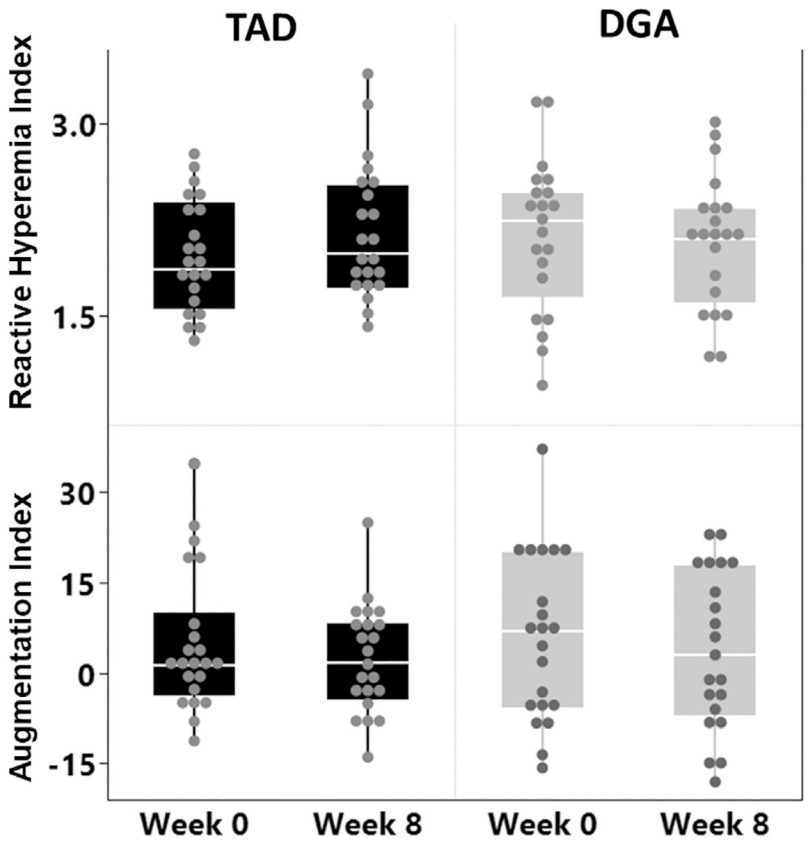

Figure 2. EndoPAT variables at weeks 0 and 8 in TAD and DGA groups:
Box-and-whisker plots, showing individual data points, at wk0 and wk8 in TAD and DGA groups. No significant differences were identified by group or week. Values are medians ± IQR.

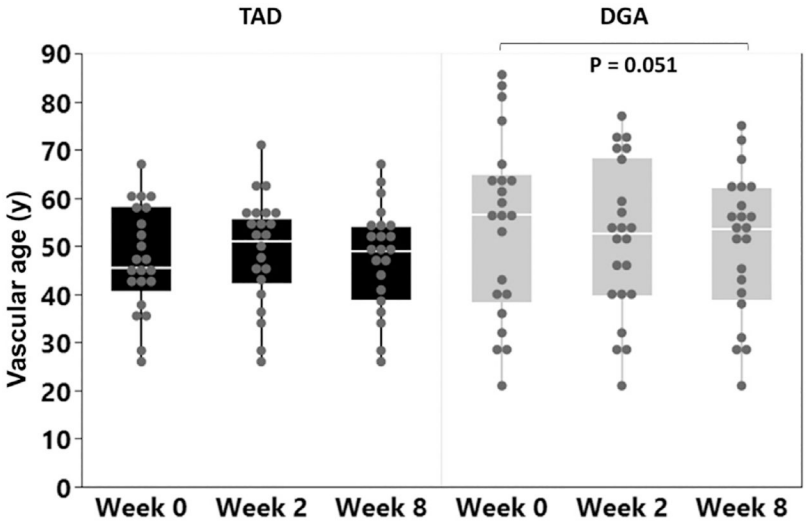

Figure 3.

Vascular age at weeks 0, 2 and 8 in TAD and DGA groups: Box and whisker plots at wks 0, 2 and 8 of both DGA and TAD interventions in women. Wk8 score following DGA intervention was lower compared to wk0 ($p = 0.051$) identified by linear mixed model analysis followed by Tukey's multiple comparison correction when a significant group × week interaction was identified ($p < 0.05$). Box and whisker plot report the median ± IQR, and individual data points for each study participant are included within the boxplots.

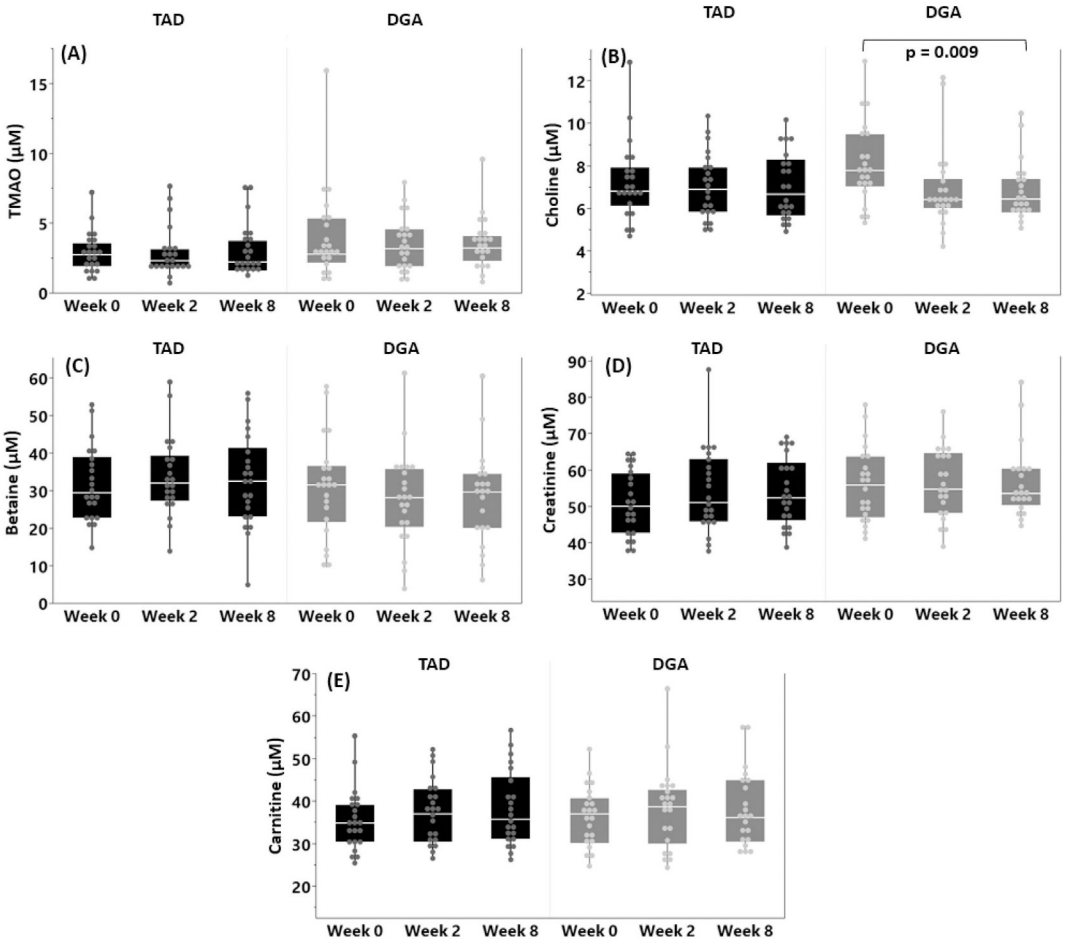

Figure 4. Plasma TMAO and related metabolites at weeks 0, 2 and 8 in TAD and DGA groups: Panels (A)–(E) depict box and whisker plots of fasting TMAO, choline, betaine, creatinine and carnitine in DGA and TAD groups at wks 0, 2 and 8 of each intervention. Panel (B) shows significant difference between choline at wk0 and wk8 ($p = 0.009$) identified by linear mixed model analysis followed by Tukey's multiple comparison correction when a significant group × week interaction was identified ($p < 0.05$). No other significant differences were identified. The box and whisker plots also show individual data points indicating circulating concentrations.

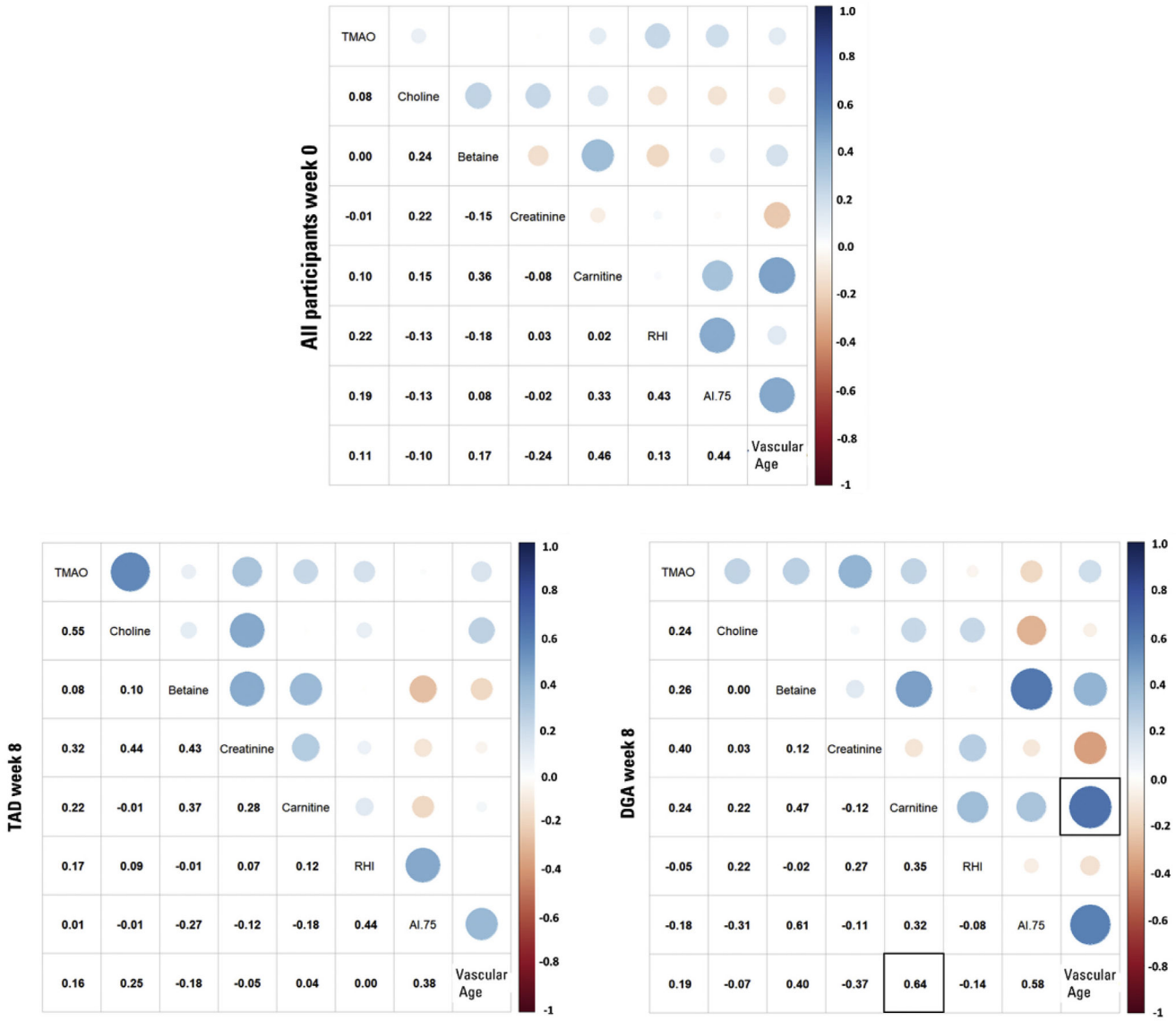

Figure 5. Spearman's rho at wk0 (n = 44) and wk8 (n = 22 DGA and 22 TAD groups) between variables of interest.

There were some associations between TMAO and related metabolites, EndoPAT variables (Reactive Hyperemia Index – RHI, and AI.75 – Augmentation Index @75 bpm heart rate) and vascular age. Positive and negative associations are indicated by blue and red color circles respectively, larger circles indicate larger rho values, the bottom half of each correlogram gives the rho values, and black boxes indicate which associations are significant ($p < 0.05$) after Benjamini-Hochberg FDR correction.