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Differential effect of short-term popular diets on TMAO and other cardio-metabolic risk markers

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Abstract *Background:* Dietary nutrient intake and its metabolism by the gut microbiome have recently been implicated in cardiovascular disease (CVD) risk. In particular, trimethylamine N-oxide (TMAO), a metabolite of the gut microbiota, has been shown to be a predictor of incident CVD events. Elevated levels of branched-chain amino acids (BCAA) have also been associated with an increased propensity for insulin resistance.
Methods: To study the association of dietary intake with systemic TMAO, its nutrient precursors, and BCAA levels on fasting plasma levels of TMAO and its nutrient precursors and BCAA, we conducted an exploratory post-hoc analysis of 3 popular diets – high fat (Atkins), Mediterranean (South Beach), and very low fat (Ornish) – using plasma samples from a prior randomized, crossover study, with each isocaloric dietary phase lasting 4 weeks. Metabolites were quantified using stable isotope dilution HPLC with on-line tandem mass spectrometry.
Results: Compared to the low fat Ornish phase, the high fat Atkins dietary phase was characterized by increased levels of TMAO (3.3 vs. 1.8 μM, $p = 0.01$), and the BCAA valine (272.8 vs. 235.8 μM, $p = 0.005$) and leucine (105.9 vs. 96.4 μM, $p = 0.01$). The high fat Atkins dietary phase was also associated with higher levels of TMAO (3.3 vs 1.6 μM, $p = 0.04$), valine (272.8 vs. 240.7 μM, $p = 0.004$), and leucine (105.9 vs. 96.4 μM, $p = 0.01$) compared to baseline.
Conclusions: These data suggest that over a 4-week interval, a saturated fat diet that is predominantly animal-based, compared to an isocaloric, low fat, predominantly plant-based diet, is associated with heightened risk for cardiometabolic derangements, as monitored by a higher plasma levels of both TMAO and BCAA.
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Background

Recent studies indicate that dietary nutrient intake and its subsequent metabolism by gut microbiota may contribute to the pathogenesis of cardio-metabolic disease [1–3]. Dietary choline, phosphatidylcholine, carnitine, and related trimethylamine containing nutrients are

Abbreviations: ANOVA, analysis of variance; BCAA, branch chained amino acids; CVD, cardiovascular disease; TMAO, trimethylamine N-oxide.

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found in a variety of meats, egg yolks, and other animal products, and are metabolized by the gut microbiota. Several metabolites, namely trimethylamine N-oxide (TMAO), as well as branched chain amino acids (BCAA) leucine, isoleucine, valine, as well as choline and betaine, have been shown to be correlated with increased cardiovascular disease (CVD) risk [3,4]. Popular diets can alter nutrient intake, changing the makeup and metabolism of gut microbiota, and may therefore alter an individual's CVD risk. In view of the significant impact that the metabolism of the gut microbiota can exert on health and the lack of comparative human data assessing these effects, we studied the effect of three popular diets – high saturated fat (Atkins); Mediterranean-style, moderate fat (South Beach); and low fat (Ornish) – to determine whether isocaloric substitution of saturated, predominantly animal-based fat would be associated with higher levels of atherogenic biomarkers compared to a low fat, predominantly plant-based diet.

Methods

Plasma samples were examined in a post-hoc exploratory analysis designed to evaluate the biological effects of three popular diets on lipids, endothelial function, and C-reactive protein during weight maintenance (Fig. 1) [5]. Briefly, 26 normolipidemic subjects, who had no history of metabolic, hepatic, renal, or systemic disease, were assigned to Atkins (high fat), South Beach (moderate fat), and Ornish (low fat) diets to examine biochemical and physiologic parameters during a period of weight maintenance. Each subject was randomly assigned to a 4-week isocaloric diet followed by a 4-week washout phase using a counterbalanced, crossover design. Inclusion criteria included body mass index <30 kg/m^2 with no metabolic, hepatic, renal, or systemic disease. Subjects were comprised of medical students, residents, and hospital employees. Use of herbal remedies, probiotics, fish oils, antibiotics, or anti-inflammatory medications was not

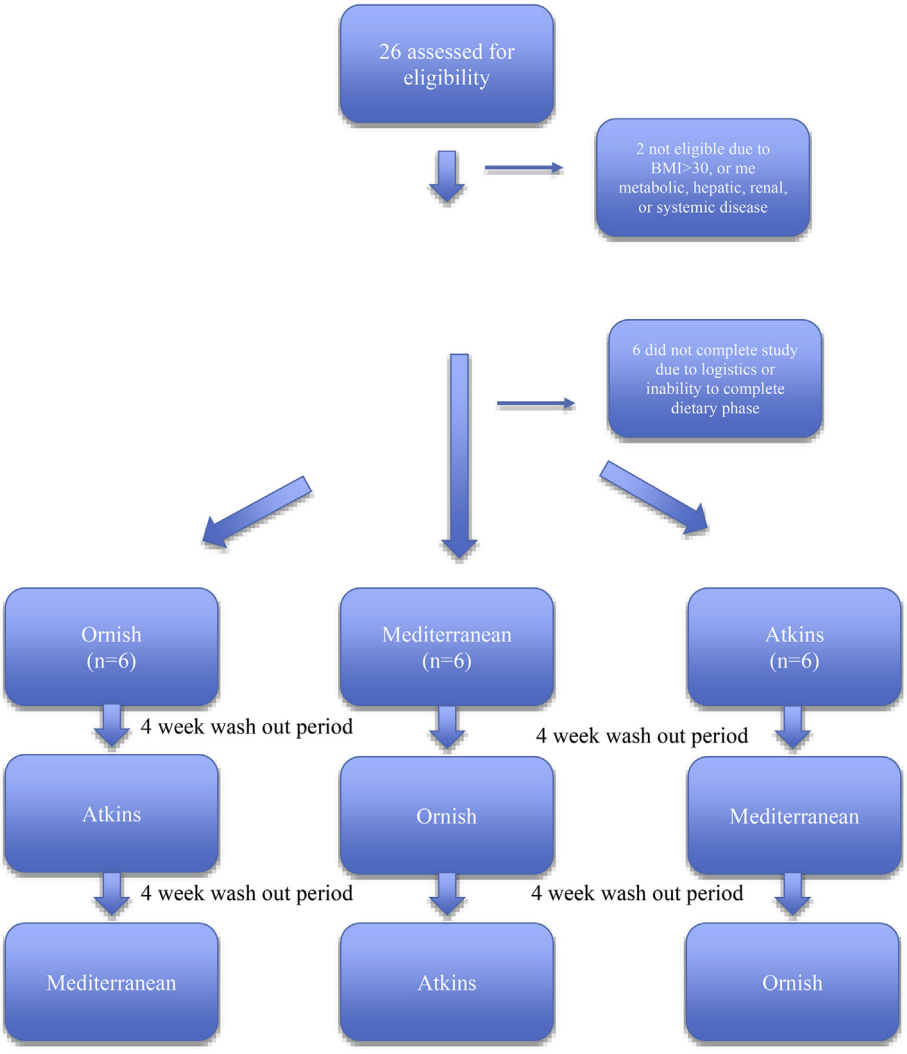

Figure 1 Consort diagram of study design. Eighteen subjects completed the original study (5). Of these, samples were available in 14 subjects to examine dietary metabolites.

permitted. Alcohol intake was permitted if <1 ounce per day. Dietary compliance was assessed through self-recorded food records that were analyzed by a registered dietitian weekly, who assessed dietary nutrient and caloric intake. Because the original study was aimed at weight maintenance, within 1–2% of body weight, subjects were weighed at weekly intervals and their diets adjusted by the dietician if there was >1 kg difference from the prior week. Eighteen subjects completed the study. Mean age was 30.6 (standard deviation [SD] 9.6) years and mean body mass index was 22.6 (3) kg/m^2, as previously reported [5]. Average caloric intake between the three diets were not measurably different among subjects, with the following average energy intake estimates: Atkins 7,216 (1844) kJ/day), South Beach 6,732 (1,338) kJ/day, and Ornish 6,869 (1,380) kJ/day. The Atkins phase was associated with higher intake of dietary cholesterol, fat, and saturated fat compared to the other two phases (see previously published data) [5]. Of those subjects, frozen plasma samples were accessible in 14 subjects each of whom completed the 3 dietary phases for a total of 42 samples available for processing. Fasting serum samples stored at −70 °C were thawed overnight in a refrigerator prior to analysis for TMAO, choline, betaine, carnitine, γ-butyrobetaine, crotenobetaine, and the individual BCAA, using stable isotope dilution HPLC with on line tandem mass spectrometry, as previously described [1,6]. All laboratory personnel were blinded to clinical results, with sample identity available only as a code. Biochemical measurements before and after each dietary phase was analyzed using STATA Statistical Data Analysis Software and SAS. Repeated measures of analysis of variance (ANOVA) was used to estimate the mean within-person change and standard error of the within-person changes in metabolic parameters. A two-tailed $p \leq 0.05$ was regarded as statistically significant.

Results

Estimated dietary intake from 72-hour food records are shown in Table 1. As noted, there were significant differences in estimated cholesterol and fat content between dietary phases. Metabolite profiles were obtained from samples from the 14 subjects (Table 2). As compared to low fat Ornish dietary phase, the high fat Atkins phase was characterized by significantly higher levels of TMAO (Atkins and Ornish mean [interquartile range {IQR}], respectively: 3.3 [2.0–4.0] vs. 1.8 [1.2–3.0] μM, p = 0.01), as well as higher levels of the branched-chain amino acids (BCAA) valine (272.8 [234.7–348.9] vs. 235.8 [209.1–246.0] μM, p = 0.005) and leucine (105.9 [97.9–125.7] vs. 95.4 [90.4–107.5] μM, p = 0.01). The high fat Atkins dietary phase was also associated with significantly higher levels of TMAO (3.3 [2.0–4.0] vs 1.6 [1.1–3.4] μM, p = 0.04), valine (272.8 [234.7–348.9] vs. 240.7 [211.0–289.2] μM, p = 0.004), and leucine (105.9 [97.9–125.7] vs. 96.4 [88.0–107.6] μM, p = 0.01) compared to baseline. In addition, the Atkins phase was associated with higher valine levels compared to the moderate fat South Beach phase (272.8 [234.7–348.9] vs. 222.5 [192.3–278.0] μM, p = 0.004) but no statistically significant difference was seen in the levels of TMAO (3.3 [2.0–4.0] vs. 2.6 [1.4–5.0] μM, p = 0.7) or leucine (105.9 [97.9–125.7] vs. 94.8 [82.4–117.6] μM, p = 0.1). Additionally, there were no significant differences among the aromatic amino acids, phenylalanine (Atkins vs. Ornish and Atkins vs. baseline, respectively [60.7 {53.7–62.5} vs. 72.3 {66.5–79.7} μM, p = 0.8], [60.7 {53.7–62.5} vs. 58.7 {56.8–61.1} μM, p = 1]) and tyrosine (64.3 [57.7–78.1] vs. 72.3 [66.5–79.7] μM, p = 0.8; 64.3 [57.7–78.1] vs. 84.2 [74.0–92.0] μM, p = 0.09).

Finally, levels of choline, carnitine, crotenobetaine or γ-butyrobetaine were not statistically significant when compared to baseline or between the different dietary phases. The Ornish phase was associated with higher levels of betaine compared to baseline (57.8 [36.0–66.2] vs. 47.1 [31.5–51.6] μM, respectively, p = 0.02). Overall, dietary compliance for each phase was excellent and consistent with the macronutrient composition as prescribed for the Atkins, South Beach and the Ornish diet (Table 1) [5].

Table 1 Selected dietary variables following exposure to popular diets.

	Atkins	South Beach	Ornish
Wt (kg)			
Pre	69.1 ± 13.4	69.0 ± 13.3	69.1 ± 13.3
Post	68.9 ± 13.3	68.9 ± 13.4	68.6 ± 12.8
KJ/day	7216 ± 1844	6732 ± 1338	6869 ± 1380
Chol (mg)	567 ± 267	202 ± 186	114 ± 89
Fiber(g)	16 ± 3[a]	26 ± 8	32 ± 16
% Energy			
Protein	26 ± 6[a]	22 ± 6	22 ± 5
Carb[b]	17 ± 8	48 ± 12	70 ± 9
Fat[b]	58 ± 9	31 ± 11	9 ± 3

[a] Significant differences compared to the 2 other dietary phases ($P < 0.05$).
[b] Significant differences between the groups ($P < 0.001$).

Discussion

In this isocaloric crossover dietary intervention study, significantly higher levels of TMAO were observed during a short-term high-saturated animal fat dietary phase (Atkins) compared to a low fat, predominantly plant-based diet (Ornish). While prior studies have shown the effect of a Mediterranean diet on fecal short-chain fatty acids [7,8], this is the first study to show a higher level of plasma TMAO in subjects consuming high fat versus low fat diets. This likely reflects, in part, the increased intake of L-carnitine, a trimethylamine abundant in red meat, and phosphatidylcholine, found predominantly in egg yolk, which serve as substrates for TMAO formation by gut microbiota [1,3]. Recent studies suggest that TMAO correlates with atherosclerotic plaque size and predicts major CVD events (death, myocardial infarction, stroke) independent of traditional CVD risk factors [1–4]. Indeed, in a recent meta-analyses of 17 studies involving cumulatively

Table 2 Metabolic profile during three diets.

	Baseline	IQR	Atkins	IQR	South Beach	IQR	Ornish	IQR
Dietary metabolites								
Choline (μM)	6.3	(5.6–7.6)	6.1	(5.7–6.5)	6.3	(5.7–7.5)	6.3	(5.1–7.6)
Betaine (μM)	47.1	(31.5–51.6)	41.6	(32.5–56.3)	49.3	(36.7–56.1)	*57.8	(36.0–66.2)
Carnitine (μM)	37.9	(32.5–46.4)	36.6	(33.0–41.3)	34.7	(33.1–47.3)	36.3	(30.8–44.4)
Butyrobetaine (μM)	0.9	(0.8–1.2)	1.0	(0.9–1.2)	1.0	(0.7–1.2)	1.0	(0.8–1.1)
Crotonobetaine (μM)	0.10	(0.08–0.11)	0.09	(0.08–0.11)	0.09	(0.08–0.13)	0.12	(0.07–0.13)
Trimethylamine N oxide (μM)	1.6	(1.1–3.4)	*3.3	(2.0–4.0)	2.6	(1.4–5.0)	**1.8	(1.2–3.0)
Amino acids								
Leucine (μM)	96.4	(88.0–107.6)	*105.9	(97.9–125.7)	94.8	(82.4–117.6)	**95.4	(90.4–107.5)
Iso-Leucine (μM)	55.1	(47.1–65.3)	54.0	(50.4–71.5)	50.3	(42.7–67.6)	54.4	(52.2–57.7)
Valine (μM)	240.7	(211.0–289.2)	*272.8	(234.7–348.9)	**222.5	(192.3–278.0)	**235.8	(209.1–246.0)
Phenylalanine (μM)	58.7	(56.8–61.1)	60.7	(53.7–62.5)	60.0	(57.2–66.6)	61.9	(59.4–64.8)
Tyrosine (μM)	84.2	(74.0–92.0)	64.3	(57.7–78.1)	80.0	(68.6–95.5)	72.3	(66.5–79.7)

*$p \leq 0.05$ compared to baseline.
**$p \leq 0.05$ compared to Atkins diet.

over 26,000 subjects, the relative risk for all-cause mortality increased 7.6% per each 10 μM increment of TMAO and persisted in all examined subgroups and across all populations examined [4]. Additionally, recent studies have shown that TMAO levels are correlated with thrombotic event risk in subjects, suggesting a reduction in thrombosis potential of a predominantly plant-based diet [9,10]. Consequently, the current analysis supports TMAO as a novel target for monitoring CVD risk assessment through dietary intervention [11]. Interestingly, prior animal studies have shown that chronic carnitine ingestion can induce production of TMAO and that vegans and vegetarians produce less TMAO from carnitine. This supports alteration of the catabolic capacity of gut microbiota as a potential future target for intervention [1]. Nonetheless, a recent study by Bogiatzi et al. suggests differential metabolic function of gut microbiota as the etiology of lower levels of TMAO in association with decreased carotid plaque burden even after controlling for coronary risk factors, despite the lack of difference in carnitine intake [12]. This study, combined with the findings of our study, suggest a more complex pathway for diet vis-à-vis functionality of the gut microbiome.

Higher levels of betaine were found in the Ornish phase as compared to baseline. Recent studies have shown that elevated choline and betaine levels are associated with increased CVD risk only when there is also an associated increase in TMAO [13].

In addition to TMAO, elevations in valine and leucine, two BCAA, were also observed during the high fat Atkins phase. Although each dietary phase was only 4 weeks long and therefore too short to predict any long-term consequences, prior studies have found increased levels of BCAA (leucine, isoleucine, valine) to be associated with reduced insulin sensitivity that is independent of obesity [14,15].

This study has several limitations. Given the small sample size and post-hoc nature of this exploratory analysis, there was no pre-specified hierarchy of endpoints or correction for multiple testing. Similarly, data on genetic variants were not available. Thirdly and by trial design, the between group comparisons are more important that within group comparisons and therefore the within group effects should be interpreted with caution. Fourthly, adherence to dietary therapy was based on self-reported dietary food records rather than having meals prepared in a metabolic kitchen. While specific foods were not analyzed to provide an estimate of the amount of TMAO present, macronutrient intake served as a proxy for the relative proportion of fat consumed in the popular diets tested. It is also notable that age, renal function (e.g., glomerular filtration rate) and genetic variants (e.g., flavin monooxygenase) affecting the microbiota may influence TMAO variance beyond nutrition-based factors [16]. Finally, the study was not included in clinicaltrials.gov as the original study was initiated prior to this requirement.

Conclusions

The current analysis demonstrates that short-term exposure to a high saturated, predominantly animal fat-based diet compared to a predominantly plant-based diet is associated with changes in the gut microbiota-derived metabolite TMAO, which has shown to be atherogenic. It also reveals relatively short term dietary changes (4 weeks) are sufficient to reduce elevated TMAO levels. The extent to which atherogenic metabolites provide incremental CVD risk beyond traditional risk factors warrants further exploration.

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Availability of data and material

The data analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JEP contributed to data analysis and was the major contributor in writing the manuscript. MM contributed to

the idea, data analysis, and editing the manuscript. JR aided in sample preparation and review and editing of the manuscript. ZW ran and analyzed the samples, performed the data analysis, and contributed to editing of the manuscript. SH conceived the idea and contributed to editing the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare that they have no competing interests. Written informed consent was obtained from all subjects prior to participation. Drs. Hazen and Wang are named as co-inventors on patents held by the Cleveland Clinic relating to cardiovascular diagnostics and therapeutics. Drs. Hazen and Wang report being eligible to receive royalty payments for inventions or discoveries related to cardiovascular diagnostics or therapeutics from Cleveland Heart Lab, and P&G. Dr. Hazen is a paid consultant for P&G; and has received research funds from P&G, Pfizer Inc., and Roche Diagnostics.

Ethics approval and consent to participate

This study was approved by the University of Maryland Institutional Review Board.

Consent for publication

Not applicable.

Acknowledgements

Not applicable.

References

[1] 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.

[2] Tang WHW, Wang Z, Levison BS, Koeth RA, Britt EB, Fu X, et al. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. N Engl J Med 2013;368:1575–84.

[3] 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.

[4] Schiattarella GG, Sannino A, Toscano E, Giugliano G, Gargiulo G, Franzone A, et al. Gut microbe-generated metabolite trimethylamine-N-oxide as cardiovascular risk biomarker: a systematic review and dose-response meta-analysis. Eur Heart J 2017;38:2948–56.

[5] Miller M, Beach V, Sorkin JD, Mangano C, Dobmeier C, Novacic D, et al. Comparative effects of three popular diets on lipids, endothelial function, and C-reactive protein during weight maintenance. J Am Diet Assoc 2009;109:713–7.

[6] Org E, Blum Y, Kasela S, Mehrabian M, Kuusisto J, Kangas AJ, et al. Relationships between gut microbiota, plasma metabolites, and metabolic syndrome traits in the METSIM cohort. Genome Biol 2017;18:70.

[7] De Filippis F, Pellegrini N, Vannini L, Jeffery IB, La Storia A, Laghi L, et al. High-level adherence to a Mediterranean diet beneficially impacts the gut microbiota and associated metabolome. Gut 2016;65:1812–21.

[8] Mitsou EK, Kakali A, Antonopoulou S, Mountzouris KC, Yannakoulia M, Panagiotakos DB, et al. Adherence to the Mediterranean diet is associated with the gut microbiota pattern and gastrointestinal characteristics in an adult population. Br J Nutr 2017;117:1645–55.

[9] Zhu W, Gregory JC, Org E, Buffa JA, Gupta N, Wang Z. Gut microbial metabolite TMAO enhances platelet hyperreactivity and thrombosis risk. Cell 2016;165. https://doi.org/10.1016/j.cell.2016.02.011.

[10] Zhu W, Wang Z, Tang WHW, Hazen SL. Gut microbe-generated trimethylamine N-Oxide from dietary choline is prothrombotic in subjects. Circulation 2017;135:1671.

[11] Brown JM, Hazen SL. Targeting of microbe-derived metabolites to improve human health: the next frontier for drug discovery. J Biol Chem 2017. https://doi.org/10.1074/jbc.R116.765388.

[12] Bogiatzi C, Gloor G, Allen-Vercoe E, Reid G, Wong RG, Urquhart BL, et al. Metabolic products of the intestinal microbiome and extremes of atherosclerosis. Atherosclerosis 2018;273:91–7.

[13] Wang Z, Tang WHW, Buffa JA, Fu X, Britt EB, Koeth RA, et al. Prognostic value of choline and betaine depends on intestinal microbiota-generated metabolite trimethylamine-N-oxide. Eur Heart J 2014;35:904–10.

[14] Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E, et al. Metabolite profiles and the risk of developing diabetes. Nat Med 2011;17:448–53.

[15] Tai ES, Tan MLS, Stevens RD, Low YL, Muehlbauer MJ, Goh DLM, et al. Insulin resistance is associated with a metabolic profile of altered protein metabolism in Chinese and Asian-Indian men. Diabetologia 2010;53:757–67.

[16] Canyelles M, Tondo M, Cedó L, Farràs M, Escolà-Gil JC, Blanco-Vaca F. Trimethylamine N-oxide: a link among diet, gut microbiota, gene regulation of liver and intestine cholesterol homeostasis and HDL function. Int J Mol Sci 2018;19(10).