

Gut Microbe-Generated Trimethylamine *N*-Oxide From Dietary Choline Is Prothrombotic in Subjects

We previously showed gut microbial production of trimethylamine *N*-oxide (TMAO) from dietary nutrients like choline, lecithin, and *L*-carnitine is linked to the development of cardiovascular diseases.¹⁻³ We also recently reported that plasma TMAO levels are associated with incident thrombotic event risk in subjects, and that TMAO both enhances platelet responsiveness to multiple agonists by augmenting stimulus-dependent Ca²⁺ signaling and heightens thrombosis potential in animal models.⁴ Specifically, a role for TMAO and gut microbiota in transmitting heightened thrombosis potential *in vivo* was supported by both direct TMAO infusion and microbial transplantation studies.⁴ A Western diet, rich in choline, is associated with heightened thrombosis risk; however, the effect of dietary choline on TMAO and platelet hyperresponsiveness in human subjects has not yet been reported.

We prospectively recruited healthy vegans/vegetarians (*n*=8) and omnivores (*n*=10) with no preceding (1-month) history of antibiotics or probiotics. This single-center study was approved by the Cleveland Clinic Institutional Review Board. After informed consent, subjects (46±5 years of age, 40% male, nonsmokers without hypertension, diabetes mellitus, or cardiovascular disease) were given oral choline supplementation (choline bitartrate 500 mg twice daily, ≈450 mg total choline/day) for 2 months with monthly blood testing after overnight fast. Both vegan/vegetarian and omnivore alike showed significant >10-fold increases in plasma TMAO levels at both 1- and 2-month periods (*P*<0.01 each; Figure, A), with corresponding enhanced platelet aggregation responses to submaximal adenosine diphosphate (5μM) after choline supplementation (Figure, A). Moreover, a striking dose-dependent association was observed between plasma TMAO levels and platelet function (Figure, B). Similarly, among all subjects in the study, a significant association was noted between change from baseline in TMAO level and change from baseline in platelet aggregation (Spearman rho=0.38, *P*=0.03).

We next tested whether platelet hyperresponsiveness associated with choline supplementation and elevated TMAO was observed in the presence of aspirin. Omnivores previously examined in the absence of aspirin had a choline supplement-free washout period of at least 1 month and then were started on aspirin (81 mg each evening) for 1 month before a baseline evaluation, followed by 2 months of choline supplementation. Compared with baseline, choline again increased both fasting plasma TMAO levels and adenosine diphosphate-dependent platelet aggregation responses at 1 and 2 months of supplementation; however, both the degree of TMAO elevation and platelet hyperresponsiveness were attenuated by aspirin therapy (Figure, C).

These studies show for the first time a direct prothrombotic effect of dietary choline and elevated levels of the gut microbial metabolite TMAO in humans. They also suggest the platelet hyperresponsiveness mediated by elevated TMAO can be attenuated by a low dose of aspirin. It is important to note that they suggest elevated levels of the gut microbe-generated metabolite TMAO may overcome the antiplatelet effects of low-dose aspirin—a hypothesis that warrants further investigation,

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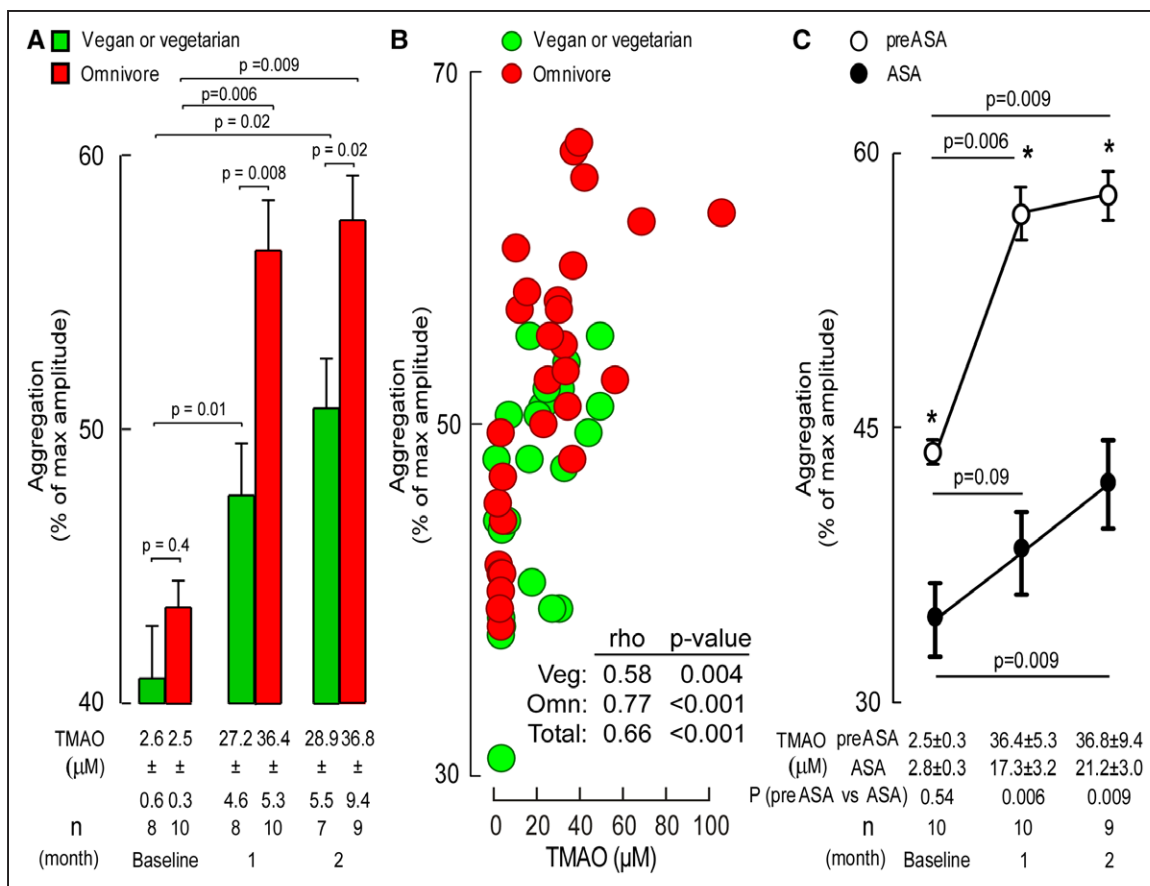


Figure. Oral choline supplementation increases fasting trimethylamine *N*-oxide (TMAO) levels, enhances platelet aggregation, and attenuates the antiplatelet effect observed with aspirin.

A, Plasma TMAO levels and platelet aggregation in response to submaximal adenosine diphosphate (5 μM) in vegan/vegetarian and omnivore groups. **B**, Correlation between plasma TMAO and platelet aggregation responses among the indicated groups. Spearman correlations and *P* values shown. **C**, Effect of choline supplementation on TMAO and platelet aggregation responses in omnivores in the absence versus presence of aspirin (ASA). All data shown are mean (\pm SEM) with the indicated number of subjects. Asterisks shown represent *P*<0.05 for comparison of aggregation responses off versus on ASA for the corresponding time point. *P* values were calculated with Wilcoxon rank sum test for two-group comparisons and Wilcoxon signed rank test for pairwise comparisons.

particularly in subjects at high cardiovascular risk. An unanticipated finding was that low-dose aspirin partially reduced choline supplement-dependent rise in TMAO. Although the mechanism for this result is unknown, aspirin has been reported to alter the composition of the gut microbial community.⁵ Finally, aspirin use in primary prevention subjects has recently been debated. The present studies, coupled with published studies linking heightened TMAO levels with thrombotic event risk,⁴ suggest studies are warranted to explore if low-dose aspirin is beneficial among subjects with elevated TMAO and no clear contraindications to aspirin.

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DISCLOSURES

Drs Hazen and Wang are named as coinventors on pending and issued patents held by the Cleveland Clinic relating to cardiovascular diagnostics and therapeutics. Dr Hazen is a paid consultant for Esperion and P&G; has received research funds from P&G, Pfizer Inc., Roche Diagnostics, and Takeda; and also reports he may receive royalty payments for inventions or discoveries related to cardiovascular diagnostics or therapeutics from P&G, Cleveland HeartLab, Siemens, Esperion, and Frantz Biomarkers, LLC. Dr Wang reports he may receive royalty payments for inventions or discoveries related to cardiovascular diagnostics or therapeutics from Cleveland HeartLab. The other authors report no conflicts of interest.

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FOOTNOTES

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