Dietary Free Choline Supplements, but not Whole Eggs, Raise Fasting TMAO Levels in Subjects with Normal Renal Function: A Randomized Clinical Trial

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Included in this online supplement:

Supplemental Methods

Authentication of Biological Sources of Choline Content. To authenticate the equivalency in supplemental total choline content provided by each intervention arm, the total choline content of whole, grade large, hardboiled eggs (Gordon Food Services, Wyoming, MI), the whites of those eggs, independent of the yolks, choline bitartrate tablets (500 mg tablets, Nature's Way, Green Bay WI), and phosphatidylcholine capsules (420 mg softgel, from soy lecithin, Swanson Health Products, Fargo ND), were independently quantified and confirmed following base hydrolysis by stable isotope dilution LC/MS/MS, as previously described, at our laboratory.[3,30]

The demonstrated total choline content per day for each intervention arm of the study is as follows: (i) four 50 g hardboiled eggs correspond to 467 mg total choline content (116.8 mg total choline per egg); (ii) two 500 mg choline bitartrate tablets correspond to 411 mg total choline content (205.5 mg total choline per tablets); (iii) four 50 g hardboiled eggs and two 500 mg choline bitartrate tablets corresponds to 878 mg total choline content; (iv) the whites of four 50 g hard-boiled eggs and two 500 mg choline bitartrate tablets corresponds to 413 mg total choline content (0.5 mg total choline per egg white); and (v) six 420 mg phosphatidylcholine capsules correspond to 410 mg total choline content (68.3 mg total choline per capsule).

Randomization Protocol. Subjects were randomized to one of four different interventions using simple randomization without stratification. A random number generator was used to assign participants to an intervention at the time of enrollment. The research coordinator was therefore blinded to the intervention arm until the day of consent. Subjects were enrolled until 10 subjects in each of the four arms had

additionally consented to the platelet sub-study and completed the protocol. After this condition was satisfied enrollment was stopped. Subjects in the PC supplements arm were enrolled after interim analyses and after the other arms had finished enrollment, so were not randomized.

eTable 1. Changes in Choline, Carnitine, and Betaine Plasma Levels from Baseline to End-of-Study in Each Treatment Group

		Arm 1 Whole Eggs	**Arm 2** Choline Tablets	**Arm 3** Whole Eggs + Choline Tablets	**Arm 4** Egg Whites + Choline Tablets	**Arm 5** PC Capsules
N		*18*	*20*	*16*	*18*	*10*
Choline (µM)	Baseline	8.3 (6.6-10.2)	7.5 (6.5-9.3)	8.5 (7.6-11.2)	9.5 (7.6-11.6)	7.6 (6.7-9.1)
	Day 28	10.9 (8.9-12.4)	12.9 (10.8-16.0)	14.0 (11.7-15.7)	12.8 (10.2-14.1)	10.6 (9.2-11.6)
	p-value	*0.005**	*< 0.001**	*< 0.001**	*0.009**	*0.04**
Carnitine (µM)	Baseline	19.1 (16.3-22.8)	21.2 (16.6-25.1)	21.5 (16.8-24.8)	21.1 (16.5-26.6)	23.4 (17.8-28.1)
	Day 28	19.4 (13.4-27.9)	18.7 (15.5-21.0)	15.6 (10.9-19.4)	18.9 (15.2-23.2)	20.8 (19.1-27.5)
	p-value	*0.93*	*0.01**	*0.003**	*0.04**	*0.99*
Betaine (µM)	Baseline	28.1 (23.6-48.3)	38.2 (28.7-48.7)	30.7 (18.4-35.7)	38.7 (23.3-47.7)	33.6 (23.3-45.0)
	Day 28	39.7 (29.4-55.5)	69.0 (54.4-82.2)	46.9 (33.9-67.5)	59.8 (41.6-65.6)	46.3 (28.3-64.0)
	p-value	*0.02**	*< 0.0001**	*< 0.0001**	*0.001**	*0.02**

* denotes significance

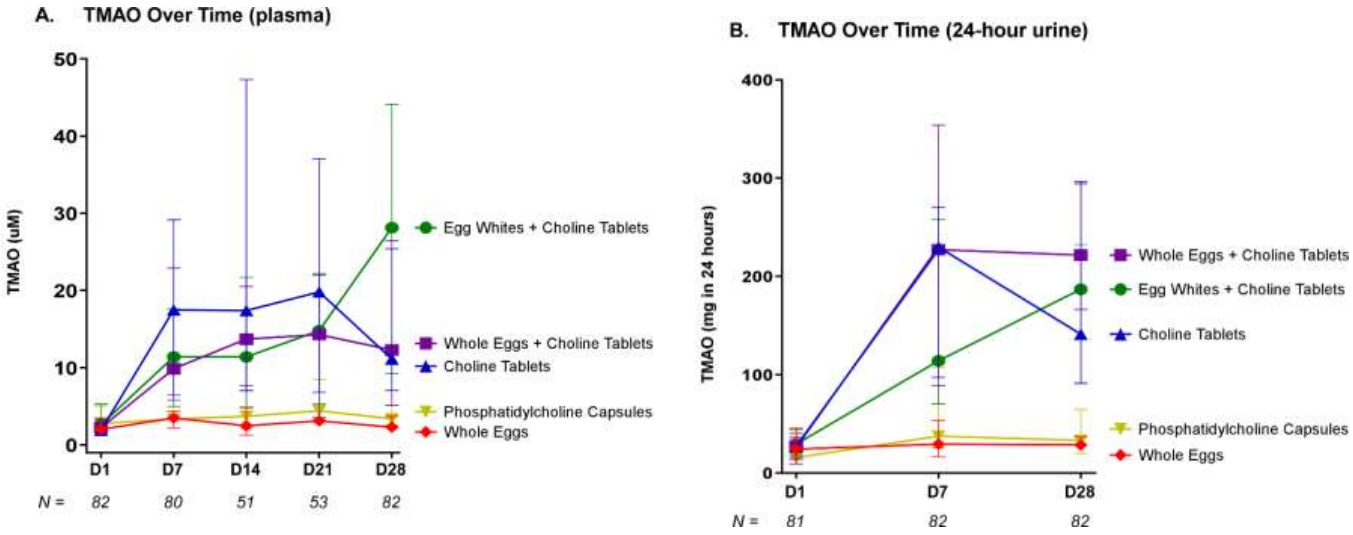
Subjects followed one of five interventions for 4 weeks. The interventions provided supplemental dietary choline from varied sources, as noted. Blood was collected at baseline and then weekly thereafter throughout the study, as outlined in the methods. Shown here are fasting plasma concentrations of choline, carnitine, and betaine observed at baseline and end of study (Day 28) for each arm. Values are reported as median (Q1-Q3). P values were calculated with the Wilcoxon rank-sum test, with values less than 0.05 deemed significant.

eTable 2. Change in Average Cholesterol Values from Baseline to End-of-Study in Each Treatment Group

		Arm 1 Whole Eggs	**Arm 2** Choline Tablets	**Arm 3** Whole Eggs + Choline Tablets	**Arm 4** Egg Whites + Choline Tablets	**Arm 5** PC Capsules
N		*18*	*20*	*16*	*18*	*10*
Total Choline Provided (mg)		486	411	897	411	410
Total Cholesterol (mg/dL)	Baseline	156 (145-175)	180 (147-201)	187 (172-204)	186 (150-189)	175 (159-217)
	Day 28	158 (136-180)	172 (157-191)	198 (168-221)	178 (157-193)	172 (151-213)
	p-value	*0.94*	*0.93*	*0.32*	*0.91*	*0.85*
Triglycerides (mg/dL)	Baseline	86 (70 - 112)	106 (74 - 134)	103 (89 - 125)	122 (52 - 156)	74 (67 - 89)
	Day 28	100 (57 - 116)	96 (82 - 141)	97 (80 - 111)	109 (69 - 170)	84 (67 - 96)
	p-value	*0.87*	*0.99*	*0.47*	*0.70*	*0.73*
HDL (mg/dL)	Baseline	48 (44 - 66)	49 (40 - 60)	57 (50 - 65)	48 (39 - 63)	61 (49 - 65)
	Day 28	49 (44 - 65)	51 (44 - 60)	56 (49 - 74)	50 (41 - 57)	62 (42 - 64)
	p-value	*0.90*	*0.75*	*0.58*	*0.92*	*0.97*
LDL (mg/dL)	Baseline	91 (72 - 102)	90 (80 - 124)	108 (98 - 120)	104 (86 - 124)	107 (90 - 137)
	Day 28	86 (71 - 110)	94 (82 - 119)	118 (87 - 132)	101 (87 - 121)	106 (79 - 136)
	p-value	*0.89*	*0.84*	*0.29*	*0.96*	*0.91*

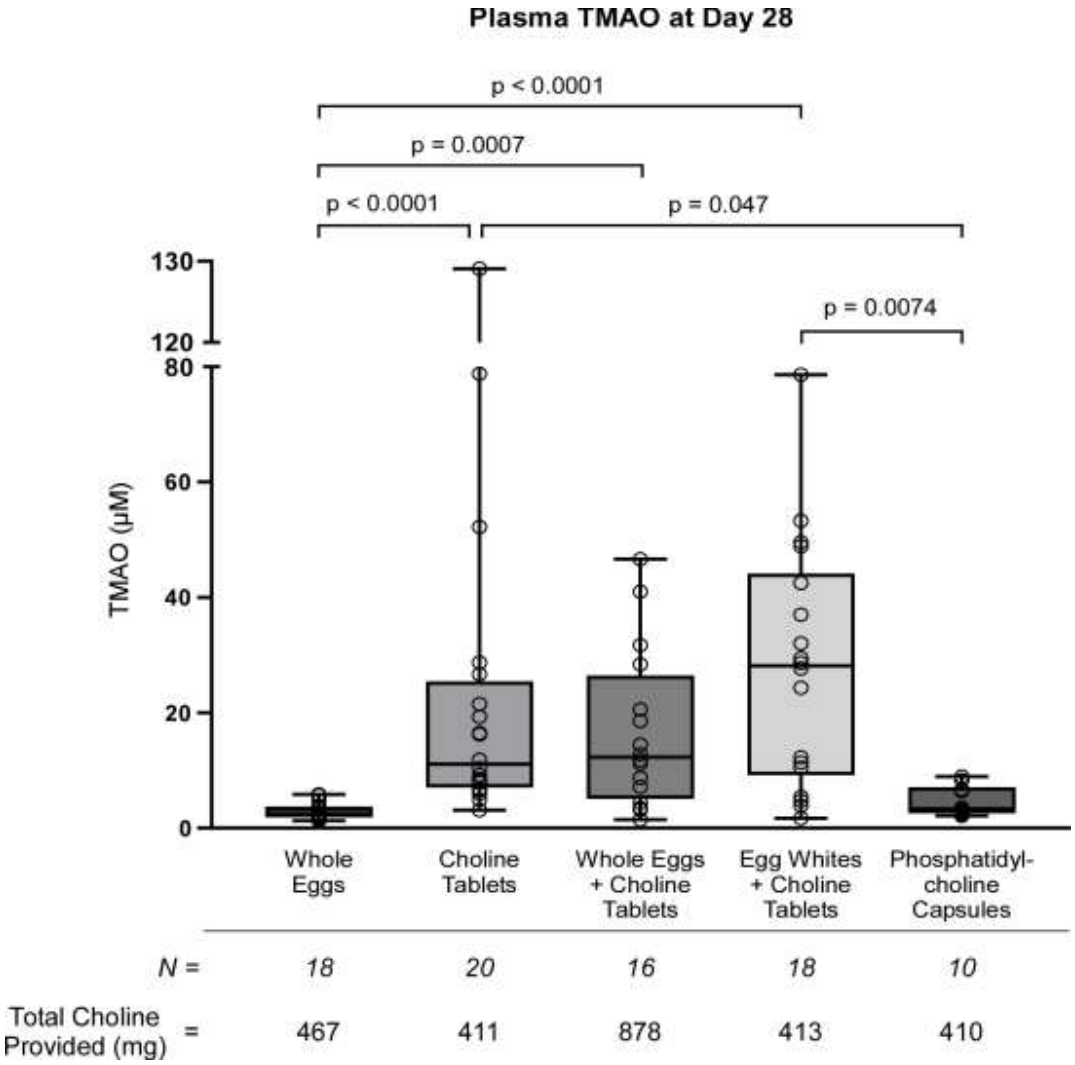
Subjects followed one of five interventions for 4 weeks. The interventions provided supplemental dietary choline from varied sources, as noted. Blood was collected at baseline and then weekly thereafter throughout the study, as outlined in the methods. Shown here are fasting plasma lipid profile levels observed at baseline and end of study (Day 28) for each arm. Values are reported as median (Q1-Q3). P values were calculated with the Wilcoxon rank-sum test, with values less than 0.05 deemed significant. HDL = high-density lipoprotein; LDL = low-density lipoprotein; PC = phosphatidylcholine. To convert total cholesterol, HDL, and LDL to mmol/L, multiply values by 0.0259. To convert triglycerides to mmol/L, multiply by 0.0113.

eFigure 1. TMAO Changes over 28 Days of Dietary Intervention



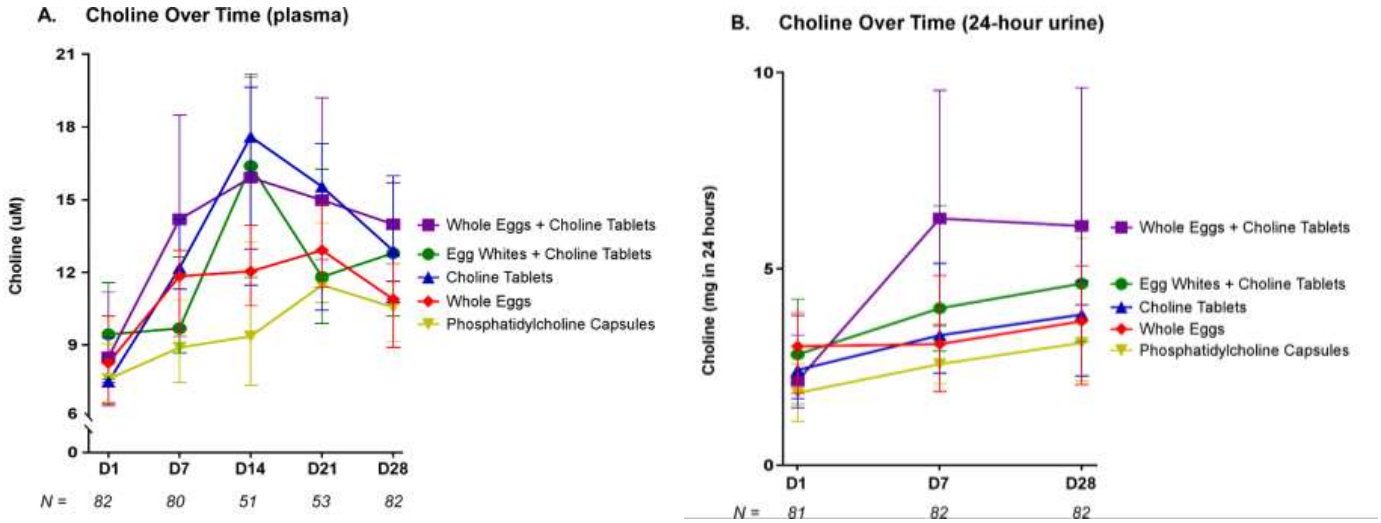
A. Fasting plasma TMAO (µM) measured at each study visit, as described under Methods. Plotted as median + IQR. **B**. TMAO production (as mg in 24-hour urine collection) measured at each study visit, mirroring the results in plasma showing a rise in TMAO concentration after only 1 week of supplementation. Plotted as median + IQR. D1 = Day 1, D7 = Day 7, D14 = Day 14, D21 = Day 21, D28 = Day 28.

eFigure 2. TMAO Differences Between Study Arms at Day 28



Comparison of fasting plasma TMAO concentrations (µM) at Day 28, the final study visit, between all study arms is shown with an indication of significant differences. The specific source of supplemental choline provided to subjects is indicated below each bar. P values were calculated with the Kruskal–Wallis test, with values less than 0.05 deemed significant.

eFigure 3. Choline Changes over 28 Days of Dietary Intervention



A. Fasting plasma choline (µM) measured at each study visit, as described under Methods. Plotted as median + IQR. **B**. Choline production (as mg in 24-hour urine collection) measured at each study visit, mirroring the results in plasma showing a rise in choline concentration in all arms across the study period. Plotted as median + IQR. D1 = Day 1, D7 = Day 7, D14 = Day 14, D21 = Day 21, D28 = Day 28.