Original Research

Short-term high-fat diet increases postprandial trimethylamine-N-oxide in humans

CrossMark

Nabil E. Boutagy [a,c,d], Andrew P. Neilson [b,c], Kristin L. Osterberg [a,c], Andrew T. Smithson [b], Tessa R. Englund [a], Brenda M. Davy [a,c], Matthew W. Hulver [a,c,d], Kevin P. Davy [a,c,d,*]

[a] The Department of Human Nutrition, Foods, and Exercise, 295 West Campus Dr, Virginia Tech, Blacksburg, VA 24061
[b] The Department of Food Science and Technology, 360 Duck Pond Dr, Virginia Tech, Blacksburg, VA 24060
[c] The Fralin Translational Obesity Research Center, 1981 Kraft Dr, Virginia Tech, Blacksburg, VA 24060
[d] The Metabolic Phenotyping Core, 1981 Kraft Dr, Virginia Tech, Blacksburg, VA 24060

ARTICLE INFO

ABSTRACT

The gut microbiota plays an obligatory role in the metabolism of nutrients containing trimethylamine moieties, such as L-carnitine and choline, leading to the production of the proatherogenic trimethylamine-N-oxide (TMAO). We hypothesized that a short-term, high-fat diet would increase fasting and postprandial plasma concentrations of TMAO in response to a high-fat meal challenge. Following a 2-week eucaloric control diet, 10 nonobese men (18-30 years) consumed a eucaloric, high-fat diet (55% fat) for 5 days. Plasma TMAO was measured after a 12-hour fast and each hour after for 4 hours following a high-fat meal (63% fat) at baseline and after the high-fat diet using ultraperformance liquid chromatography/ tandem mass spectrometry. Fasting plasma TMAO did not increase significantly following the high-fat diet (1.83 ± 0.21 vs 1.6 ± 0.24 μmol/L). However, plasma TMAO was higher at hour 1 (2.15 ± 0.28 vs 1.7 ± 0.30 μmol/L), hour 2 (2.3 ± 0.29 vs 1.8 ± 0.32 μmol/L), hour 3 (2.4 ± 0.34 vs 1.58 ± 0.19 μmol/L), and hour 4 (2.51 ± 0.33 vs 1.5 ± 0.12 μmol/L) (all $P < .05$) following the high-fat diet as compared with the baseline postprandial response. In conclusion, a short-term, high-fat diet does not increase fasting plasma TMAO concentrations but appears to increase postprandial TMAO concentrations in healthy, nonobese, young men. Future studies are needed to determine the mechanisms responsible for these observations.

1. Introduction

Cardiovascular disease is the leading cause of death in the United States [1] and worldwide [2]. Recently, the gut microbiota has been implicated in the initiation and progression of cardiovascular disease [3]. Rodent and human studies have revealed a meta-organismal pathway in which the gut microbiota plays an obligatory role in the metabolism of nutrients

containing trimethylamine (TMA) moieties (eg, L-carnitine, choline, and betaine) that lead to the production of TMA, which is subsequently oxidized by hepatic flavin monooxygenase 3 (FMO3) to trimethylamine-N-oxide (TMAO). Trimethylamine-N-oxide has been hypothesized to increase forward cholesterol transport by upregulating macrophage scavenger receptors [4,5]. Subsequently, the increase in cholesterol uptake in the vascular wall leads to macrophage foam cell formation and atherosclerotic development [4,5]. In addition, in large prospective epidemiological studies, TMAO is independently associated with the incidence of major cardiovascular events and cardiovascular disease mortality [5,6].

Choline and L-carnitine are nutrients that are primarily present in animal-based high-fat foods. Long-term dietary intake influences the capacity of the gut microbiota to produce TMA and, consequently, TMAO from these nutrients. In rodents, continuous intake of L-carnitine–supplemented chow increases the synthetic capacity of the gut microbiota to produce TMA 10-fold [5]. Similarly, human omnivores have a greater capacity to produce TMA and TMAO following an L-carnitine meal challenge compared with vegans [5]. In addition, omnivores have higher proportions of bacterial genera that are associated with fasting plasma TMAO concentrations as compared with vegans. Notably, the gut microbiota can rapidly switch (in as little as 1 day) between herbivorous and carnivorous functional profiles in response to low-fat, plant-based and/or high-fat, animal-based diets [7]. However, whether a short-term, high-fat diet increases fasting and postprandial plasma concentrations of TMAO in response to a high-fat meal challenge containing TMA moieties is unknown.

Accordingly, we hypothesized that a short-term, high-fat diet would increase fasting and postprandial plasma TMAO concentrations in response to a high-fat meal challenge. To test our hypothesis, we measured plasma TMAO concentrations after a 12-hour fast and each hour after for 4 hours following a high-fat meal (63% fat) at baseline and following a short-term, high-fat diet using ultraperformance liquid chromatography (UPLC)–tandem mass spectrometry (MS/MS). The plasma samples were obtained from subjects who were included in a larger study that examined the effects of a high-fat diet on skeletal muscle metabolism endpoints. Our goal was to obtain proof-of-concept for larger mechanistic studies.

2. Methods and materials

2.1. Materials

The following were obtained from Sigma-Aldrich: TMAO (catalog 317594), L-carnitine hydrochloride (catalog 588105), choline chloride (catalog C7017), betaine chloride (catalog B2629), choline-d_9 chloride (catalog 492051), and betaine-d_9 chloride (catalog 616656). All other supplies were purchased from the following: TMAO-d_9 (Cambridge Isotope Laboratories, catalog DLM47791), L-carnitine-d_9 (Cambridge Isotope Laboratories, catalog DLM-3555-PK), and UPLC solvents acetonitrile (ACN) (VWR, catalog JT9829-3) water (VWR, catalog JT9831-3).

2.2. Subjects

Ten nonobese (body mass index, 18-30 kg/m^2) men (18-30 years old) who were included in a larger study examining the

effects of a high-fat diet on skeletal muscle metabolism end points comprised the study sample. We excluded women in this initial investigation to eliminate potential confounding due to the transient trimethylaminuria (elevated urinary TMA) that has been reported to occur during menstruation [8]. Participants were weight stable (±2.5 kg), sedentary to recreationally active (≤2 days, 20 min/d of low-intensity physical activity), and abstained from antibiotic use for at least 6 months before study commencement. All participants were normotensive (blood pressure <140/90 mm Hg), normoglycemic (fasting glucose <100 mg/dL), and normolipemic (total cholesterol <200 mg/dL, triglycerides <150 mg/dL). Participants were not taking any medications or supplements (eg, prebiotics/probiotics) that could influence variables at the time of the study and were free from overt chronic diseases as determined by health history, blood chemistry, and urinalysis. In addition, participants were excluded if their total daily fat consumption was ≥40% and/or their total daily saturated fat consumption was ≥15% based on 4-day food recalls. A flowchart for subject selection is shown in Supplementary Fig. 1. The Virginia Polytechnic and State University Institutional Review Board approved the study protocol. The nature, purpose, risks, and benefits of the study were explained before obtaining written and verbal informed consent.

2.3. Experimental design

Following a 2-week eucaloric control diet, all participants completed baseline testing. Immediately following baseline testing, participants consumed a eucaloric, high-fat diet (55% fat) for 5 days. Participants repeated baseline testing immediately following the high-fat diet.

2.4. Controlled feeding

All diets were controlled to minimize the potential impact of interindividual variability in habitual dietary intake. Energy requirements were estimated based on height, weight, age, and activity level using the Institutes of Medicine equation [9]. Subsequently, a 7-day cycle menu was constructed for each participant with the appropriate macronutrient and caloric content for each diet (control and high fat) using Nutrition Data System for Research (NDS-R) software (University of Minnesota). Food modules (250 kcal) with the same macronutrient composition as the control and/or high-fat diet were added or subtracted if a participant's weight changed by more than 1 kg. In addition, participants were weighed each morning during the control and high-fat diet periods to ensure weight stability.

Participants consumed breakfast in the metabolic kitchen and dining laboratory of the Department of Human Nutrition, Foods, and Exercise each day and were provided a cooler with food for the remainder of the day. Participants were instructed to only consume the provided food for the duration of the study and were instructed to report all nonstudy foods, if consumed, to research staff. Participants were instructed to return any uneaten food and all unwashed food containers to monitor compliance.

For the first 2 weeks of the study, all participants consumed a eucaloric control diet (55% carbohydrate, 30% fat, 15% protein). Following the lead-in diet, all participants consumed a eucaloric,

high-fat diet for 5 days. The macronutrient composition of this diet was 55% fat (50% of which was saturated fat), 30% carbohydrate, and 15% protein. All participants consumed a high-fat meal (2 sausage, egg, and cheese biscuits) consisting of 850 kcal (63% fat [21% saturated fatty acid {SFA}], 25% carbohydrate, and 12% protein), before and following the high fat diet to assess postprandial TMAO production.

2.5. Experimental testing

All testing took place at the Human Integrative Physiology Laboratory between 5:00 AM and 11:00 AM. Participants remained 12-hours fasted (included caffeinated and alcoholic beverages), performed no vigorous physical activity for the prior 48 hours, and were free from acute illness for the prior 2 weeks.

2.6. Measurements

Body mass was measured on a digital scale (Model 5002; Scale-Tronix, Inc, Carol Stream, IL, USA), and height was measured using a stadiometer. Body composition was measured using dual-energy x-ray absorptiometry (Lunar Prodigy Advance, GE Medical Systems, software version 8.10e, Little Chalfont, United Kingdom). Habitual dietary intake was assessed using detailed 4-day diet records. Participants were instructed on the proper way to weigh and record food intake for 3 weekdays and 1 weekend day. Habitual dietary intake was analyzed with NDS-R software by a trained diet technician. The dietary composition and energy intake of the controlled diet were determined using NDS-R.

Fasting plasma concentrations of TMAO, L-carnitine, choline, and betaine were quantified by isocratic UPLC-MS/MS using the stable isotope dilution method against internal standards (IS), as described previously by Kirsch et al [10] and with modifications as described below.

The choline chloride, betaine chloride, L-carnitine hydrochloride, TMAO, betaine-d9 chloride, and choline-d9 chloride standards were obtained from Sigma (St Louis, MO, USA). The TMAO-d9 and L-carnitine-d9 standards were obtained from Cambridge Isotope Laboratories (Tewksbury, MA, USA). The UPLC solvents (ACN and water) were liquid chromatography–mass spectrometry grade (VWR, Randor, PA, USA).

Choline, betaine, and TMAO were analyzed together; and separate plasma samples were prepared for L-carnitine analysis. For analysis of choline, betaine, and TMAO, a stock solution of the 3 IS, (25.5, 26.8, and 28.0 μmol/L for betaine-d9, choline-d9, and TMAO-d9, respectively) was prepared in water and stored at −20°C. Immediately before sample preparation, the IS stock solution was diluted 100-fold with ACN (1 mL IS stock + 99 mL ACN) for final IS concentrations of 255, 268, and 280 nmol/L for betaine-d9, choline-d9, and TMAO-d9, respectively.

For analysis of L-carnitine, a stock solution of the IS (29.4 μmol/L L-carnitine-d9) was prepared in water and stored at −20°C. Immediately before sample preparation, the IS stock solution was diluted 25-fold with ACN (4 mL IS stock + 96 mL ACN) for final IS concentrations of 1.18 μmol/L L-carnitine-d9.

Plasma samples were thawed at room temperature, and 50 μL plasma was combined with 600 μL of the ACN/IS solution in a 1.5-mL microcentrifuge tube. The addition of ACN resulted in protein precipitation. Analytes were extracted by vigorous vortexing for 30 seconds. The samples were centrifuged for 3 minutes at 17 000g at room temperature. Samples were filtered using 0.2-μm Phililc PTFE Millex Samplicity vacuum filters (Millipore, Billerica, MA, USA) into Waters (Milford, MA, USA) Total Recovery LCMS Certified high-performance liquid chromatography vials and analyzed immediately by UPLC-MS/MS. The UPLC-MS/MS analyses were carried out using a Waters Acquity UPLC system coupled to a Waters TQD triple quadrupole mass spectrometer. The system software was MassLynx (Waters).

The samples were separated on a Waters BEH HILIC analytical column (2.1 × 100 mm; particle size, 1.7 μm) with a Waters BEH HILIC VanGuard precolumn (2.1 × 5 mm; 1.7 μm). The column temperature was at 30°C, and the sample compartment was maintained at 10°C. The mobile phases were 15 mmol/L ammonium formate, pH 3.5 (phase A) and ACN (phase B). The system flow rate was 0.65 mL/min, and isocratic elution was achieved using 20% A/80% B over 3 minutes. Following UPLC separation, the target analytes and their respective IS were identified and quantified using electrospray ionization in (+)-mode. The source and capillary temperatures were 150°C and 400°C, respectively. The capillary voltage was 0.60 kV, and the desolvation and cone gas (both N_2) flow rates were 800 and 20 L/h, respectively.

The compounds were quantified using multireaction monitoring (MRM) functions optimized by Intellistart, as shown in Table 1. The MRM functions used the Autodwell function to optimize the number of points per peak (12 points for a 10-second peak). The detection span was ±0.2 amu for each mass. Fig. 1 shows MRM chromatograms from a representative subject, extracted from plasma that were spiked with the IS, L-carnitine-d9, choline-d9, betaine-d9, and TMAO-d9.

Quantification was completed using QuanLynx (Waters). Quantification was performed using the ratio of the target analyte and respective IS peak areas based on external standard curves prepared using a wide range of target analyte concentrations (bracketing the peak areas observed in the plasma samples) and the same IS concentrations used to prepare the plasma samples.

2.7. Statistical analyses

All statistical analyses were performed with Prism 6 (GraphPad Software, San Diego, CA, USA). Dependent t tests were used to compare dependent variables of interest between the high-fat diet and control diet periods. A repeated-measures analysis of variance was used to examine the effect of diet, meal challenge, and diet by meal challenge interaction on the dependent variables of interest. Post hoc analyses were performed with a Sidak multiple comparison test. All of the data are expressed as means ± SE. The significance level was set a priori at $P < .05$.

3. Results

3.1. Participant characteristics

Participant characteristics at baseline and following the high-fat diet are shown in Table 2. There were no differences in body mass, body mass index, body fat percentage, body fat mass, and lean mass following the high-fat diet (all $P > 0.05$).

Table 1 – MRM functions for target analytes and IS

Compound	Retention time (min)	MW (g/mol)	Parent [M + H]$^+$ (m/z)	Daughter (m/z)	Cone voltage (V)	Collision energy (eV)
Betaine	1.25	117.15	118.24	59.42	44	18
Betaine-d9	1.25	126.14	127.3	68.10	46	18
Choline	1.13	103.16	104.2	60.02	38	16
Choline-d9	1.11	112.16	113.32	69.08	40	16
TMAO	2.01	75.11	76.16	58.91	40	10
TMAO-d9	1.98	84.12	85.22	68.1	40	12
L-Carnitine	2.09	161.20	162.26	84.99	34	20
L-Carnitine-d9	2.08	170.25	171.28	84.99	34	20

3.2. Dietary composition

The dietary composition of control and high-fat diet and the high-fat meal challenge is shown in Table 3. By design, total energy intake was similar during high-fat diet compared with the control dietary period ($P > 0.1$). In addition, total fat and SFA intakes were higher during the high-fat diet compared with the control diet (all $P < 0.01$). Carbohydrate, total fiber, and fiber per 1000 kcal/d intakes were significantly lower for high-fat diet compared with the control diet. There was no significant difference in protein intake between the diets.

3.3. Fasting and postprandial plasma TMAO concentrations

Fasting and postprandial plasma concentrations of TMAO at baseline and following the high-fat diet are shown in Fig. 2. Fasting plasma TMAO concentration was not significantly different from baseline following the high-fat diet (Fig. 2). There was no effect of the meal challenge on postprandial TMAO production at baseline (Fig. 2). However, plasma TMAO increased significantly at hour 2, hour 3, and hour 4 as compared with fasting concentrations following the high-fat diet (Fig. 2). In addition, plasma TMAO concentrations were significantly higher at hours 1 to 4 during the postprandial period following the high-fat diet compared with those time points during the control diet (all $P < 0.05$). The individual tracings for each subject's fasting and postprandial plasma TMAO concentrations at baseline and following the high-fat diet are shown in Supplementary Fig. 2.

3.4. Fasting and postprandial plasma L-carnitine, choline, and betaine concentrations

Fasting and postprandial plasma concentrations of L-carnitine, choline, and betaine at baseline and following the high-fat diet are shown in Table 4. There was no effect of the meal challenge on postprandial plasma L-carnitine and choline concentrations at baseline or following the high-fat diet. In addition, there were no differences in the postprandial
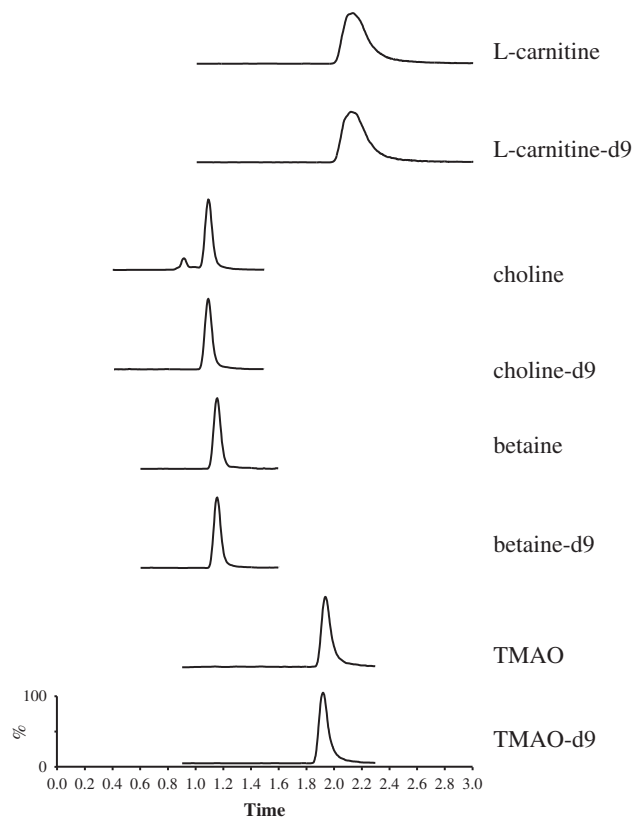
Fig. 1 – UPLC-MS/MS MRM chromatograms extracted from a representative subject's plasma spiked with IS (L-carnitine-d9, choline-d9, betaine-d9, and TMAO-d9). MRM fragment transitions ([M + H]$^+$ parent ion m/z → daughter ion m/z) are as follows: L-carnitine: 162.26 → 84.99; L-carnitine-d9: 171.28 → 84.99; choline: 104.2 → 60.02; choline-d9: 113.32 → 69.08; betaine: 118.24 → 59.42; betaine-d9: 127.3 → 68.10; TMAO: 76.16 → 58.91; TMAO-d9: 85.22 → 68.10.

Table 2 – Participant characteristics at baseline and following the high-fat diet

Variable	Baseline	High-fat diet
Age, y	22.1 ± 0.5	22.1 ± 0.5
Body weight, kg	70.4 ± 3.1	70.3 ± 3.1
Body mass index, kg/m^2	22.3 ± 1.0	22.3 ± 0.9
Body fat, %[*]	23 ± 1.0	23 ± 1.0
Body fat mass, kg[*]	15.8 ± 1.6	15.6 ± 1.6
Lean mass, kg[*]	51.8 ± 2.0	52.7 ± 2.0

Values are expressed as means ± SE. n = 10 for baseline and the high-fat diet period. Dependent t tests were used to compare mean differences between the control and high-fat diet periods.
[*] n = 8 for baseline for the high-fat diet body composition measures.

Table 3 – Energy, macronutrient, and TMA-containing micronutrient intake for the control and high-fat diets and high-fat meal challenge

|	Control diet	High-fat diet	High-fat meal
Energy, kJ/d	11,531 ± 27	11,573 ± 334	3,556
Protein, g/d	105 ± 2.0	104 ± 3.0	28
CHO, g/d	378 ± 9.0*	210 ± 6.0	54
Fat, g/d	96 ± 2.0	160 ± 5.0*	60
SFA, g/d	28 ± 1.0	75 ± 2.0*	20
Fiber, g/d	17 ± 1.0*	14 ± 1.0	2.0
Fiber, g/1000 kcal	6 ± 0.2*	5 ± 0.2	2.4

Values are expressed as means ± SE. n = 10 for control and the high-fat diet period. Dependent *t* tests were used to compare mean differences between the control and high-fat diet periods. CHO = carbohydrates.
* P < 0.01, diet effect.

concentrations of plasma ʟ-carnitine and choline (both P > 0.05) in the high-fat diet compared with control diet. Furthermore, there was no effect of the meal challenge on postprandial betaine concentrations at baseline or following the high-fat diet. However, plasma betaine concentration decreased significantly following the high-fat diet at hour 2 following the meal challenge when comparing this time point during the control diet (P < 0.05).

4. Discussion

Based on our observations, we reject our hypothesis that a short-term, high-fat diet leads to an increase in fasting plasma concentrations of TMAO. However, we accept our hypothesis that a short-term, high-fat diet leads to increases
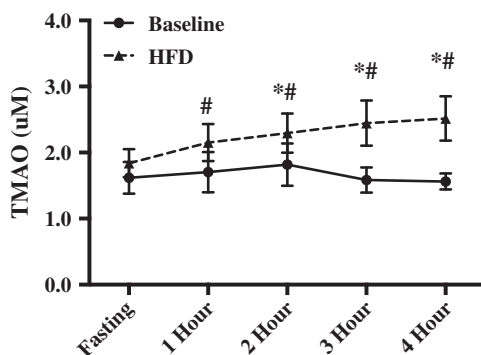
Fig. 2 – Plasma TMAO concentrations after a 12 hour fast and each hour for 4 hours following a high-fat meal (63% fat) at baseline and following a short-term (5 days) high-fat diet (55% fat). Values are expressed as means ± SE. n = 10 for baseline and the high-fat diet period. A repeated-measures analysis of variance was used to examine the effect of the diet, the meal challenge, and diet by meal challenge interaction. Post hoc analyses were performed with a Sidak multiple comparison test. *P < .05, meal effect; means are significantly different from fasting. #P < .05, meal challenge and diet interaction; means are significantly different between diet periods. ● with solid line = baseline and ▲ with dashed line = high-fat diet.

in postprandial plasma concentrations of TMAO in response to a high-fat meal challenge. In addition, plasma betaine concentration was reduced following the high-fat diet at hour 2 of the postprandial period when comparing this time point during the control diet.

The short-term, high-fat diet administered in this study did not significantly increase fasting TMAO concentrations. Adults eating a mixed diet consume between 60 and 180 mg/d of ʟ-carnitine [11], about 300 mg/d of choline [12], and 100 mg/d of betaine [13]. As such, it is possible that the relative intake of TMA-containing nutrients provided in this study was not sufficient to change the large and variable circulating TMAO pool over the short time period studied. Stella et al [14] reported that consumption of a diet high in red meat (420 g/d, 30% of total energy as fat) for 15 days increased urinary TMAO in nonobese, healthy men, thus supporting the notion that longer-term dietary interventions may be necessary to affect fasting TMAO concentrations.

The mechanism(s) by which the high-fat diet may have contributed to increases in postprandial TMAO concentrations is (are) poorly understood. Our study was not designed to address this issue, but there are several plausible mechanisms. Firstly, wide-ranging compositional and functional changes occur rapidly (eg, between 1 and 5 days) in the gut microbiota in response to either a low-fat and -protein, high-fiber (plant based) diet or a low-fiber, high-fat and -protein (animal based) diet [7]. In addition, continuous ʟ-carnitine supplementation in rodents or continuous consumption of an omnivorous diet in humans shifts the gut microbial composition to favor bacterial species that prefer TMA containing nutrients (eg, ʟ-carnitine and choline) as a fuel source [5]. This gut microbial shift subsequently leads to greater TMA and TMAO production following an ʟ-carnitine meal challenge [5]. Therefore, it is possible that the short-term, high-fat diet administered in the present study may have altered the gut microbial composition to favor the metabolism of the TMA-containing nutrients, thus leading to greater TMAO production following a meal containing these substrates. Future studies are needed to test this hypothesis. Secondly, liver FMO3 expression and activity greatly influence plasma TMAO concentrations [15]; and FMO3 expression is under complex genetic, hormonal, and dietary control [15,16]. Therefore, it is possible that FMO3 expression increased following the high-fat diet and contributed to increases in TMAO concentrations in the postprandial period. Lastly, the fractional excretion of TMAO may have been decreased following the high-fat diet [4]. Along these lines, there is a discordant relationship between the appearance of labeled (d-9) and unlabeled TMAO following ingestion of d-9 phosphatidylcholine and ʟ-carnitine [5,6]. Unlabeled plasma TMAO appears in plasma within 1 to 2 hours following a meal, whereas labeled TMAO tends to appear in plasma at least 4 hours post meal. This observed delay in the appearance of labeled TMAO is postulated to reflect the time required to form TMAO from ingested TMA [6]. Therefore, the early appearance of TMAO following a meal reflects changes in the endogenous pool of TMAO and is postulated to occur because of changes in the fractional excretion rate of TMAO [4]. As such, the increases in TMAO observed in the present study, at least in the early time points, likely reflect changes in the endogenous TMAO pool and not TMAO production.

Table 4 – Fasting and postprandial plasma concentrations of TMAO substrates pre– and post–high-fat diet

	Baseline					High-fat diet				
	Fasting	Hour 1	Hour 2	Hour 3	Hour 4	Fasting	Hour 1	Hour 2	Hour 3	Hour 4
L-Carnitine, μmol/L	11.1 ± 1.5	10.5 ± 1.2	10.2 ± 1.1	12.2 ± 1.6	10.3 ± 1.3	12.1 ± 1.6	11.1 ± 1.5	11.0 ± 1.4	10.9 ± 1.4	10.2 ± 1.3
Choline, μmol/L	10.2 ± 0.6	10.7 ± 0.6	10.7 ± 0.6	10.4 ± 0.6	10.2 ± 0.7	9.3 ± 0.6	10.0 ± 0.6	9.8 ± 0.4	9.9 ± 0.5	9.7 ± 0.6
Betaine, μmol/L	56.7 ± 8.1	59.3 ± 10.5	59.3 ± 10.5	56.8 ± 7.4	57.6 ± 6.4	43.8 ± 6.4	47.5 ± 5.7	47.2 ± 5.6 *	44.5 ± 3.4	48.6 ± 8.7

Values are expressed as means ± SE. n = 10 for baseline and the high-fat diet period. A repeated-measures analysis of variance was used to examine the effect of the diet, the meal challenge, and the diet by meal challenge interaction. Post hoc analyses were performed with a Sidak multiple comparison test.

* $P < 0.05$, diet effect.

Indeed, future studies with radiolabeled TMAO and measurements of fractional excretion rate of TMAO are needed to test this hypothesis.

There was no effect of the meal challenge on postprandial betaine concentrations at baseline or following the high-fat diet. However, we observed decreased plasma betaine concentrations following the high-fat diet at hour 2 following the meal challenge when compared with this time point before the high-fat diet. The mechanism(s) of this postprandial decrease in plasma betaine is (are) not currently known. However, it is possible that the lower betaine intake during the high-fat diet compared with the control diet led to a lower circulating betaine pool and thus contributed to lower peak postprandial plasma betaine concentrations. Future studies are needed to test this hypothesis.

There are some limitations of the present study that should be discussed. Firstly, findings in our sample population of healthy, nonobese men may not be generalizable to the general population. Secondly, we may not have been adequately powered to detect changes in fasting plasma TMAO concentrations with the high-fat diet. As such, future studies with a larger sample size will be necessary to confirm our observations. Thirdly, we did not assess 24-hour urinary TMAO and therefore may not have captured the possibility of greater total body TMAO production following the diet. Lastly, we did not assess the possible mechanisms (eg, TMA production by fecal bacteria, fractional excretion of TMAO, FMO3 expression) responsible for the kinetic changes leading to increases in TMAO in the postprandial period. Future studies will be necessary to address these possibilities.

In conclusion, a short-term, high-fat diet does not increase fasting plasma TMAO concentrations but appears to increase postprandial TMAO concentrations in response to a high-fat meal in healthy, nonobese, young men. Larger studies, including those focused on other populations, are needed to determine the mechanism(s) responsible for these observations.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.nutres.2015.07.002.

Acknowledgment

REFERENCES

[1] Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, et al. Heart disease and stroke statistics—2015 update: a report from the American Heart Association. Circulation 2015;131:e29-322.

[2] Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012;380:2095–128.

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

[4] Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, DuGar B, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nat Med 2011;472:57–63.

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

[6] Tang WW, 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.

[7] David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nat Lett 2014;505:559–63.

[8] Shimizu M, Cashman JR, Yamazaki H. Transient trimethylaminuria related to menstruation. BMC Med Genet 2007;8:1–3.

[9] Institute of Medicine of the National Academies. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids. National Academies Press. 2002 and 2005.

[10] Kirsch SH, Herrmann W, Rabagny Y, Obeid R. Quantification of acetylcholine, choline, betaine, and dimethylglycine in human plasma and urine using stable-isotope dilution ultra performance liquid chromatography–tandem mass spectrometry. J Chromatogr B 2010;878:3338–44.

[11] Rebouche CJ. Kinetics, pharmacokinetics, and regulation of L-carnitine and acetyl-L-carnitine metabolism. 2004;1033:30–41.

[12] Chester DN GJ, Ahuja JK, Moshfegh AJ. Dietary intakes of choline: what we eat in America, NHANES 2007-2008. Food surveys research group dietary data brief no. 9; 2011[http://ars.usda.gov/Services/docs.htm?docid=19476].

[13] Bidulescu A, Chambless LE, Siega-Riz AM, Zeisel SH, Heiss G. Repeatability and measurement error in the assessment of choline and betaine dietary intake: the Atherosclerosis Risk in Communities (ARIC) study. Nutr J 2009;8:1–6.

[14] Stella C, Beckwith-Hall B, Cloarec O, Holmes E, Lindon JC, Powell J, et al. Susceptibility of human metabolic phenotypes to dietary modulation. J Proteome Res 2006;5:2780–8.

[15] Bennett BJ, Vallim TQ, 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 Metab 2013;17:49–60.

[16] Warrier M, Shih DM, Burrows AC, Ferguson D, Gromovsky AD, Brown AL, et al. The TMAO-generating enzyme flavin monooxygenase 3 is a central regulator of cholesterol balance. Cell Rep 2015;10:326–38.