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RESEARCH ARTICLE

## Dietary, anthropometric, and biochemical factors influencing plasma choline, carnitine, trimethylamine, and trimethylamine-*N*-oxide concentrations

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### ABSTRACT

The objective of the study was to evaluate the nutritional, anthropometric, and biochemical factors that influence choline, L-carnitine, trimethylamine (TMA), and trimethylamine-*N*-oxide (TMAO) metabolism in elderly women. The volunteers' diet was assessed using a food frequency questionnaire. Dietary patterns were estimated using a self-established score method. Body mass index (BMI), serum glucose, total, HDL, LDL cholesterol, triacylglycerol, homocysteine (tHcy), free choline (fchol), L-carnitine, TMA, and TMAO were assessed. Higher concentrations of L-carnitine, fchol, and TMAO were found in those women who had more western-style dietary patterns. Nor choline or betaine intake affected plasma fchol, TMA, or TMAO. BMI was positively correlated with fchol and TMA. tHcy was positively correlated with fchol, TMA, and TMAO, while fchol was also positively correlated with TMA and TMAO. Dietary patterns and plasma tHcy concentration influence fchol, TMA, and TMAO plasma concentration. Plasma TMA and fchol may be associated with BMI.

### ARTICLE HISTORY

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Choline; L-carnitine; trimethylamine (TMA); trimethylamine-*N*-oxide (TMAO); dietary pattern

### Introduction

Elevated levels of trimethylamine-*N*-oxide (TMAO) were identified in 2011 as a risk factor for cardiovascular events, because they enhance cholesterol accumulation in macrophages and in foam cells in artery walls (Wang et al. 2011). TMAO is synthesized in the liver by oxidation of trimethylamine (TMA) through flavin monooxygenase enzymes (especially FMO3 and FMO1) (Mendelsohn & Larrick 2013). Much of our present knowledge of the determinants of TMA concentration is based on studies of people with trimethylaminuria (fish odor syndrome), which is a result of a mutation in the *FMO3* gene that leads to a deficiency in the FMO3 enzyme (Chalmers et al. 2006). Those people are advised to avoid dietary sources of TMA and TMAO (that is, fish and shellfish), as well as dietary precursors of TMA. Substrates such as TMAO, choline, phosphatidylcholine (PtdC), L-carnitine, and betaine (Chalmers et al. 2006; Clouatre & Bell 2013) are used by bacteria in the human gut to form TMA. TMA is not synthesized during antibiotic therapy (Tang et al. 2013). The overall formation of TMAO may depend on the bioavailability of TMA precursors for microbial conversion, gut microbiota composition,

and FMO3 activity. The bioavailability of TMA precursors depends on its concentration in food products and digestibility (Conlon & Bird 2015).

The best nutritional sources of choline include egg yolks, liver, and various kinds of meat (Patterson et al. 2008). Choline may also be found in dietary supplements. The health claims approved by the European Food Safety Authority (EFSA) for use on such supplements are “contributes to normal lipid metabolism”, “maintains healthy liver function”, and “contributes to normal homocysteine metabolism” (EFSA Panel on Dietetic Products, Nutrition and Allergies 2011). PtdC can also be synthesized in the human body from choline via the Kennedy pathway, or through sequential methylation of phosphatidylethanolamine (Reo et al. 2002). This latter reaction is catalyzed by the phosphatidylethanolamine-*N*-methyltransferase (PEMT) enzyme. The *PEMT* gene is induced by estrogen, although choline synthesis in postmenopausal women who are not treated with estrogens is deprived (Fischer et al. 2010).

The nutritional sources of L-carnitine are mostly those of animal origin, such as meat or milk; however, it can also be found in small amounts in grain products

and in dietary supplements aimed at reducing fat tissue during physical activity. L-Carnitine may also be synthesized in the body from amino acids, such as lysine and methionine. The intake of carnitine correlates with its plasma concentration, and people consuming greater amounts of meat products have higher concentrations of L-carnitine than do vegetarians (Steiber et al. 2004).

The major sources of betaine include whole grains, beetroot, and spinach (Patterson et al. 2008). Betaine may also be synthesized in the body through the irreversible oxidation of choline in the liver and the kidneys via a two-step process. Betaine may function as a methyl donor in homocysteine methylation reactions, forming methionine. The betaine and folate metabolic pathways are thought to be interrelated, as homocysteine may also be methylated by methionine synthase, which uses a methyl group from 5-methyltetrahydrofolate (Craig 2004). High plasma homocysteine concentrations have been shown to be a marker of cardiovascular disease (Ganguly & Alam 2015).

Cardiovascular disease (CVD) is one of the leading causes of death worldwide, and women after menopause are at increased risk of CVD. Although TMAO has been identified as a risk factor for CVD, the interrelation between its concentration and other risk factors (such as overweight, lipid profile, and unbalanced diet) has not been well elucidated. Experimental data, mainly load tests, on the dietary determinants of TMA and TMAO do exist (Zhang et al. 1999; Wang et al. 2011; Tang et al. 2013); however, results from observational studies are scarce. Cardiovascular disease risk and TMAO concentration increases with age (D'Agostino et al. 2008; Wang et al. 2014) and higher values of TMAO are observed in men and in people with diabetes (Obeid et al. 2016).

In postmenopausal women, choline intake strongly contributes to the choline pool, as they have a reduced capacity to synthesize choline. We thus hypothesized that, in elderly women, diet may strongly affect plasma choline and its metabolites – that is, TMA and TMAO. The aim of the present study was thus to evaluate selected nutritional, anthropometric, and biochemical factors that influence choline, L-carnitine, TMA, and TMAO metabolism in elderly women.

## Methods and materials

### Subjects and study design

The study participants were originally recruited to another study aimed at evaluating the impact of folic acid supplementation (400 µg/d for 8 weeks) on serum tHcy levels and lipid metabolism biomarkers (Chmurzynska et al. 2013). The research protocol was

approved by the Local Ethics Committee (560/09) at Poznań University of Medical Sciences. All measurements in the present study were performed prior to the supplementation with folic acid. A group of female volunteers over 60 years of age was recruited from the University of the Third Age (UTA) and from a publicly run nursing home in Poznań, Poland. The exclusion criteria in the original study were diabetes mellitus, cancer, treatments interfering with folate metabolism, megaloblastic anemia, and regular intake of folic acid supplements. All participants gave their informed written consent.

### Anthropometric measurements

Body weight, body mass index (BMI, calculated as body weight (kg)/height squared (m<sup>2</sup>)), waist circumference, hip circumference, and waist-to-hip ratio (WHR) were assessed. The subjects were dressed in light clothing during the measurements, which were recorded to the nearest 0.5 cm and 0.5 kg. Body mass and height were measured with an electronic scale with a stadiometer (Seca, Warsaw, Poland).

### Food intake

Daily intakes of food products, groups of food products, choline levels, and betaine levels were assessed using a food frequency questionnaire (FFQ) based on the FFQ published by Pufulete et al. (2002) and modified for the Polish population. The questionnaire was validated in the Polish population. The FFQ included food products that are major sources of folates, choline, betaine, and carnitine. The choline and betaine content was calculated based on the USDA Database for the Choline Content of Common Foods (Patterson et al. 2008), since no such information is available in Polish food composition tables. Equivalent products to those found in Poland were chosen for calculations. Participants whose consumption was below the median value were placed in a “low intake” (LI) group, whereas those whose consumption was above the median were placed in a “high intake” (HI) group. Moreover people who had high intake of at least 2 of the following nutrients: folates, choline, and betaine, were placed into a high methyl donor intake group.

### Dietary pattern estimation

To estimate whether individuals have a more prudent or western dietary pattern, a score method was used. People in the HI group received 1 point for each of: whole grain bread, whole grain cereal, vegetables, fruits, nuts and seeds, low fat dairy products, poultry,

fish, and juice. Those who consumed high amounts of sweet breakfast cereals, white bread, pork, processed meat, fruit drinks, or confectionary lost 1 point for each such product. Those who received at least 5 points (that is, above the median) were placed into the prudent dietary pattern group while those who received less than 5 points were placed into the western dietary pattern group.

### Blood parameters

Overnight fasting blood samples were collected for biochemical parameter assessment. Plasma and serum were stored at  $-20^{\circ}\text{C}$  prior to analysis.

Plasma TMA, TMAO, and free choline (fchol) concentrations were determined by ultra-high-performance liquid chromatography electrospray ionization mass spectrometry (RP-UHPLC-ESI-MS) analysis performed using a Dionex UltiMate 3000 UHPLC (Thermo Fisher Scientific, Sunnyvale, CA) coupled to a Bruker maXis impact ultrahigh resolution orthogonal quadrupole-time-of-flight accelerator (qTOF) equipped with an ESI source and operated in positive ion mode (Bruker Daltonik, Bremen, Germany), according to the method described by Johnson (2008).

The plasma tHcy concentration was measured using high-performance liquid chromatography according to the method described by Bald et al. (2004). Plasma L-carnitine concentrations were assessed using enzymatic colorimetric assay kit (BioVision Inc., Milpitas, CA) according to protocol provided by the supplier. Serum glucose, total cholesterol, HDL cholesterol, and triacylglycerol (TAG) concentrations were measured by enzymatic colorimetric assay using a Vitalab Flexor biochemical analyzer (Vital Scientific, Spankeren, Netherlands). LDL cholesterol was calculated using the Friedewald equation. Serum folate concentrations were measured on an AxSYM system (Abbott, Abbott Park, IL).

### Statistical analysis

Differences in biochemical parameters between the LI and HI groups were analyzed using Student's *t*-test for independent variables. The results are presented as relative differences (RDs) in the concentration of biochemical parameters between the HI and LI groups.

Where log, square root, and reciprocal transformations did not impact the normality of distributions, the nonparametric Mann-Whitney *U*-test was performed (plasma carnitine). To estimate the relationship between the biochemical parameters and the selected nutritional factors, correlation coefficients and

BMI-adjusted partial correlation coefficients were calculated (Pearson's for the parametric variables and Spearman's for the nonparametric variable – that is, plasma L-carnitine).  $p < .05$  was taken to indicate statistical significance. Data were analyzed using Statistica software (version 8.0; Statsoft Inc., Tulsa, OK).

### Results

One hundred and twenty-two female volunteers ( $68.5 \pm 7.4$  years of age) participated in the study. The mean concentrations of the examined biochemical parameters and the values of the anthropometric parameters, with their standard deviations, are given in Table 1.

#### Determinants of L-carnitine concentration

Several dietary factors influenced L-carnitine concentrations (Table 2). Higher concentrations were seen in people with low intake of whole grain cereals, legumes, alcohol, or confectionary. Moreover, people with lower than the adequate intakes of choline ( $< 425$  mg/d) had higher concentrations of plasma L-carnitine ( $24.83 \pm 1.26$   $\mu\text{M}$  versus  $18.01 \pm 1.09$   $\mu\text{M}$ ). Plasma L-carnitine was inversely correlated with the intake of betaine ( $r = -.19$ ,  $p < .05$ ) and with the dietary pattern score ( $r = -.21$ ,  $p < .05$ ) (Table 2).

A relationship was observed between plasma L-carnitine concentration and lipid profile and glucose concentration (Table 3). Spearman correlation coefficients were 0.25 for total cholesterol, 0.22 for LDL cholesterol, 0.20 for triacylglycerol, and 0.24 for glucose concentration ( $p < .05$ ). BMI did not influence plasma L-carnitine concentration. However, after adjustment for BMI, a statistically significant correlation was observed between plasma L-carnitine and hip circumference ( $r = .20$ ).

#### Determinants of plasma fchol concentration

People with the western-style dietary pattern had higher concentrations of plasma fchol ( $2.95 \pm 0.09$   $\mu\text{M}$ ) than those with the prudent dietary pattern ( $2.74 \pm 0.06$   $\mu\text{M}$ ) (Table 2). Higher concentrations of plasma fchol were also recorded in people from the HI group for white bread and eggs and from the LI group for legumes, mushrooms, cheese, yoghurt, kefir, buttermilk, and dried fruits (only a trend toward significance being observed in case of milk products and dried fruits). Choline and betaine intake did not affect fchol concentrations, but intake of folate or methyl donors did. People from the LI group for folates and

**Table 1.** Mean concentrations of biochemical parameters, values of anthropometric parameters, and intake of choline, betaine, and folate with their standard deviations (SDs) in the entire study group of elderly women and showing the division into western-style and prudent dietary patterns.

Parameter	Mean $\pm$ SD		
	Whole group	People with prudent dietary pattern	People with western-style dietary pattern
Body weight (kg)	68.7 $\pm$ 10.9	69.2 $\pm$ 11.1	67.9 $\pm$ 10.8
Age	68.5 $\pm$ 7.5	68.4 $\pm$ 6.7	68.7 $\pm$ 8.5
BMI (kg/m <sup>2</sup> )	26.68 $\pm$ 4.14	26.7 $\pm$ 4.1	26.6 $\pm$ 4.3
Waist circumference (cm)	85.9 $\pm$ 9.7	85.4 $\pm$ 9.3	86.7 $\pm$ 10.2
Hip circumference (cm)	104.9 $\pm$ 8.0	105.1 $\pm$ 7.9	104.6 $\pm$ 8.4
WHR	0.82 $\pm$ 0.06	0.81 $\pm$ 0.06	0.83 $\pm$ 0.07
Total cholesterol (mg/dl)	238.6 $\pm$ 41.3	236.0 $\pm$ 40.0	242.5 $\pm$ 43.3
HDL (mg/dl)	71.0 $\pm$ 17.6	71.4 $\pm$ 15.6	70.4 $\pm$ 20.6
LDL (g/dl)	144.2 $\pm$ 34.6	142.3 $\pm$ 34.6	147.0 $\pm$ 34.9
TG (mg/dl)	118.6 $\pm$ 51.3	113.0 $\pm$ 49.9	127.4 $\pm$ 52.8
Glc (mg/dl)	84.8 $\pm$ 11.1	85.4 $\pm$ 11.4	83.9 $\pm$ 10.7
tHcy ( $\mu$ M)	7.94 $\pm$ 2.99	7.71 $\pm$ 2.71	8.29 $\pm$ 3.38
L-carnitine ( $\mu$ M)	23.27 $\pm$ 11.41	21.51 $\pm$ 9.66	25.97 $\pm$ 13.33
TMA ( $\mu$ M)	0.88 $\pm$ 0.59	0.88 $\pm$ 0.51	0.89 $\pm$ 0.71
TMAO ( $\mu$ M)	14.04 $\pm$ 2.36	13.67 $\pm$ 2.17	14.61 $\pm$ 2.55
fchol ( $\mu$ M)	2.82 $\pm$ 0.55	2.74 $\pm$ 0.50	2.95 $\pm$ 0.62
Choline intake (mg/d)	392.0 $\pm$ 25.6	438.6 $\pm$ 331.0	320.3 $\pm$ 167.4
Betaine intake (mg/d)	115.1 $\pm$ 4.4	121.7 $\pm$ 51.2	105.1 $\pm$ 43.0
Folate intake ( $\mu$ g/d)	282.5 $\pm$ 11.3	330.5 $\pm$ 126.7	208.5 $\pm$ 76.6

**Table 2.** Relative differences (RD) in plasma L-carnitine, fchol, TMA, and TMAO concentrations between people of high and low intake of selected groups of food products and nutrients. Differences between people with prudent and western dietary patterns are also shown.

Food group	RD L-carnitine	RD fchol	RD TMA	RD TMAO
Breakfast cereals, including	-10.4	-2.0	-11.7 <sup>t</sup>	-0.8
Sweet breakfast cereals	10.4	-7.1	8.1	-1.7
Whole grain cereals	-18.8*	0.3	-1.9	-1.1
Bread, including	8.0	4.0	35.8	1.2
White bread	10.5	8.8*	3.0	7.5*
Whole grain bread	-8.6	-6.0 <sup>t</sup>	21.1	-1.5
Rice, grains, pasta	-11.2	-2.5	-15.8	-1.4
Potatoes	16.9	1.6	15.3	4.6
Vegetables, including	-12.2	-2.4	-5.0	-4.3
Legumes	-9.1*	-6.9*	-14.3 <sup>t</sup>	-3.8
Salad vegetables (tomato, peppers, and onion)	-10.1	-5.7	-21.5 <sup>t</sup>	-6.4*
Mushrooms	-1.7	-8.9*	-21.3*	-5.2 <sup>t</sup>
Fruits	-5.8	-4.8	-20.5 <sup>t</sup>	-7.9*
Nuts, seeds	-12.2	-4.9	6.1	-0.8
Dried fruits	-13.6	-6.1 <sup>t</sup>	-2.6	-5.8*
Eggs	-11.0 <sup>t</sup>	8.2*	4.3	0.5
Milk products, including	-7.7	-1.4	-17.2 <sup>t</sup>	-0.8
Cheese	-11.7	-6.2 <sup>t</sup>	-11.6	-8.1*
Milk	10.8 <sup>t</sup>	7.2 <sup>t</sup>	-5.5	3.6
Yoghurt, kefir, buttermilk	-10.1	-5.7 <sup>t</sup>	-6.2	-5.8*
Meat products	4.8	3.3	25.6*	1.6
Fish	-8.1 <sup>t</sup>	-4.3	2.4	-4.0
Alcohol	-17.6*	-4.3	2.4	-2.4
Confectionary	-11.3*	-4.4	-2.4	3.9
Choline supplements	-20.2	-5.0	17.6	-8.8 <sup>t</sup>
Dietary pattern	-17.2	-7.1*	-1.2	-6.4*
Folates	-9.3 <sup>t</sup>	-7.0*	-14.2*	-5.6 <sup>t</sup>
Choline	5.2	1.1	2.2	-2.7
Betaine	-11.1 <sup>t</sup>	-5.2	-4.9	-2.5
Methyl donors	-3.9	-7.1*	-12.8*	-6.7*

RD >0: people with high intake have higher concentration; RD <0: people with low intake have higher concentration.

\*Significant with  $p < .05$ .

<sup>t</sup>Trend  $0.05 < p < .10$ .

**Table 3.** Correlation coefficients between plasma L-carnitine (Spearman), fchol, TMA, and TMAO (Pearson) concentrations and the intake of selected nutrients, dietary pattern score, anthropometric and biochemical parameters (\* $p < .05$ ).

	L-Carnitine	fchol	TMA	TMAO
Dietary pattern score	-0.21*	-0.13	-0.09	-0.27*
Folates	-0.26*	-0.16	-0.05	-0.18
Choline	-0.17	-0.00	-0.05	-0.03
betaine	-0.19*	-0.16	-0.11	-0.07
BMI	0.15	0.24*	0.21*	-0.01
Waist circumference	0.07	0.19	0.16	0.05
Hip circumference	0.15	0.16	0.14	-0.06
WHR	-0.04	0.11	0.09	0.12
Total cholesterol	0.25*	-0.04	0.00	-0.16
HDL	-0.02	-0.18	-0.22*	-0.14
LDL	0.22*	-0.02	0.05	-0.11
TG	0.20*	0.17	0.19	-0.06
Glc	0.24*	0.07	0.14	-0.01
Folic acid	0.02	0.03	-0.17	-0.14
tHcy	-0.04	0.23*	0.30*	0.21*
TMAO	-0.14	0.38*	0.18	1.00
TMA	-0.09	0.35*	1.00	0.18
fchol	-0.01	1.00	0.35*	0.38*

for methyl donors had higher concentrations of plasma fchol than those from the corresponding HI groups.

Plasma fchol concentrations were positively correlated with BMI, tHcy, TMA, and TMAO concentrations (the Pearson correlation coefficients were 0.24, 0.23, 0.38, and 0.35, respectively). They were not correlated with intake of any of the examined nutrients (Table 3).

### Determinants of plasma TMA concentrations

Higher plasma TMA concentrations were observed among people from the HI group for meat products and among people from the LI group for mushrooms (Table 2). A statistical trend toward significantly higher TMA concentrations was observed for people of LI of breakfast cereal, legumes, salad vegetables, fruits and milk products. People with high intakes of folates or methyl donors had lower TMA concentrations. BMI, tHcy, and fchol were positively correlated with TMA ( $r = .21, .30, \text{ and } .35$ , respectively), whereas HDL cholesterol and TMA were negatively correlated ( $r = -.22$ ) (Table 3).

### Determinants of plasma TMAO concentrations

As with fchol, people with the western-style dietary pattern had higher concentrations of plasma TMAO ( $14.61 \pm 0.37 \mu\text{M}$ ) than those with the prudent dietary pattern ( $13.67 \pm 0.25 \mu\text{M}$ ) (Table 2). Intakes of products that affected fchol concentrations also influenced TMAO concentrations in the same manner. Those products were white bread (higher concentration in

the HI group), mushrooms (a trend toward significance being observed for higher concentration in LI group), cheese (higher concentration in the LI group), yoghurt, kefir, and buttermilk (higher concentration in the LI group), and dried fruits (higher concentration in the LI group). Moreover, higher concentrations of TMAO were observed among people of LI for salad vegetables and fruits. People with higher intake of methyl donors had lower levels of TMAO (Table 3). There was no relationship between the anthropometric parameters and TMAO concentration, but a positive correlation of TMAO with tHcy and fchol was observed ( $r = .21$  and  $r = .38$ , respectively). After adjusting for BMI, the correlation between TMAO and fchol was higher ( $r = .40$ ), but remained the same for the other variables.

### Discussion

This observational study showed that dietary habits and patterns influence plasma fchol and carnitine, as well as the concentrations of their gut metabolites (TMA, TMAO). People with the western-style dietary pattern are more likely to have higher concentrations of TMAO, which is a risk factor for cardiovascular disease. Moreover, we showed that TMAO concentration is correlated with tHcy, which is another marker of cardiovascular disease.

This study showed that fchol plasma concentration was not affected by choline intake. Similar results were also obtained by other researchers (Sanchez et al. 1984; Wiedeman et al. 2015). Moreover, studies have indicated that different forms of ingested choline – such as free choline, glycerophosphocholine, phosphocholine, phosphatidylcholine, and sphingomyelin, as well as total choline intake – are not associated with plasma fchol values (Wiedeman et al. 2015). Konstantinova et al. (2008) also showed that egg and legumes consumption influenced fchol concentrations. Our study showed that the more western-style the dietary pattern, the higher the plasma fchol; however, Konstantinova et al. did not show any such association. This might be because of different methodology used to assess dietary patterns (principal component analysis versus self-established dietary pattern score).

Several studies (Olthof & Verhoef 2005; Cho et al. 2006; Chiuve et al. 2007; Lee et al. 2010) indicate that higher choline and betaine intakes are associated with lower plasma tHcy concentrations. The present study showed that tHcy correlated with folate, betaine, and methyl donor intakes (data not shown), but not with choline intake. Although fchol was not correlated with choline intake, it was positively correlated with tHcy

and BMI. Similar results were found by others (Konstantinova et al. 2008). The western-style dietary pattern may be the reason for the observed association, because people with a higher western dietary pattern score had higher plasma fchol, and studies have indicated that this type of dietary pattern is related to hyperhomocysteinemia (Weikert et al. 2005), cardiovascular disease risk (Stewart et al. 2016), and obesity (Paradis et al. 2009).

We showed that L-carnitine metabolism may be connected with glucose and lipid metabolism, because plasma L-carnitine was positively correlated with serum total cholesterol, LDL cholesterol, triacylglycerol, and glucose. Carnitine supplementation studies in both humans and animals have demonstrated an improvement in glucose tolerance, in particular during insulin-resistant states (Ringseis et al. 2012). In this study, we showed the opposite association; however, people with diabetes were excluded from our research. The effect of carnitine supplementation may be modulated by the body weight of an individual, and Galloway et al. (2011) indicated that carnitine supplementation lowered glucose level in lean patients and increased it in obese individuals. In this study, most people were overweight (45.1%) or obese (15.6%), which may explain the result.

Observational studies of dietary factors affecting trimethylamine concentration are lacking. An experimental study conducted by Zhang et al. (1999) showed that the intake of choline, carnitine, and TMAO, as well as most fish and seafoods, increased the urinary excretion of TMA and TMAO. However, that study considered only acute responses. The TMA concentration may depend not only on food intake over the last day, but also on dietary habits established during longer period of time, as well as on the composition of gut microbiota and concentration of food constituents which may modulate gut microbiota composition (Conlon & Bird 2015). Although this study did not show that dietary patterns impact TMA concentrations, it did show that people consuming smaller amounts of food products abundant in dietary fiber – and also those consuming lower amounts of milk products – had higher concentrations of plasma TMA. One possible explanation of the result is that such a diet favors the growth of TMA-producing bacteria (Romano et al. 2015).

To our knowledge, this is the first study to have indicated a positive correlation of TMA concentration with BMI and tHcy and a negative correlation with HDL cholesterol. These associations indicate that TMA concentration may be linked to obesity and risk of cardiovascular disease. That the composition of gut

microbiota in obese people (Mathur & Barlow 2015) and in people with cardiovascular diseases (Karlsson et al. 2012) differ from healthy controls may explain this association of TMA with anthropometric and biochemical parameters.

The study group of elderly women had a high mean concentration of plasma TMAO, at  $14.04 \pm 2.36 \mu\text{M}$  (from  $8.21 \mu\text{M}$  to  $22.78 \mu\text{M}$ ). The median plasma TMAO concentrations among the participants in other studies on people of similar ages were  $4.36 \mu\text{M}$  (Obeid et al. 2016) and  $3.9 \mu\text{M}$  ( $2.6\text{--}6.3 \mu\text{M}$ ) (Wang et al. 2011). Research shows that elevated plasma TMAO is associated with more advanced left ventricular diastolic dysfunction (Tang et al. 2015), coronary atherosclerosis (Stubbs et al. 2016), and chronic kidney disease (Mafune et al. 2016). About 44% of participants in this study were taking antihypertension drugs, while 75.4% had improper lipid profiles (total cholesterol, HDL, LDL cholesterol, and triacylglycerol in serum).

This study showed that the overall dietary pattern, as well as the intake of several dietary products, affected plasma TMAO concentrations. People with western-style dietary patterns had higher concentrations of plasma TMAO. This result is in line with other research (De Filippis et al. 2015), which showed that low adherence to a Mediterranean diet is associated with higher TMAO levels in the urine. Although TMAO has been found to be a biomarker of meat, fish, and meat-containing diets in several studies (Scalbert et al. 2014), this study did not find any such association. Several studies have shown that red meat consumption in particular is associated with TMAO production (Stella et al. 2006; Koeth et al. 2013). Elderly people in this group preferred eating poultry to other meat products. Beef was consumed in low amounts (mean intake of beef among beef consumers: 7.9 g/d) by 30% of people. We also did not show any influence of egg intake on TMAO concentration, as was demonstrated in the research of Miller et al. (2014). However, they did conduct research in an experimental environment where the participants ate up to 6 egg yolks and the acute response was measured. The minimum number of egg yolks that raised the TMAO was 2. In our study, participants ate 0–2 eggs per day and we measured the chronic state. To our knowledge, the only studies investigating the relationship between the diet and plasma L-carnitine were comparisons between vegetarian and omnivore dietary patterns and experimental studies on the influence of carnitine supplementation on various health characteristics. The higher concentrations of L-carnitine in omnivores can be explained not only by the higher intake of carnitine sources (meat and milk), but also by

the higher intake of amino acids such as methionine and lysine, which are L-carnitine precursors (Krajcovicová-Kudláčková et al, 2000). Although we did not estimate the methionine or lysine intake, we observed that the intake of products abundant in methionine and carnitine (from meat) did not influence L-carnitine concentrations. However, elderly people in this group preferred eating poultry, which has the lowest concentration of L-carnitine of all meat products.

We showed that TMAO concentration is positively correlated ( $r = .21$ ) with tHcy – one of the markers of cardiovascular disease (Ganguly & Alam 2015). Obeid et al. (2016) also showed that TMAO is associated with one carbon metabolism, as in their study it was not only correlated with tHcy ( $r = .174$ ) and fchol ( $r = .318$ ), but also with folates ( $r = -.202$ ). A higher concentration of fchol leads to a higher production of TMAO and consequently the lower use of choline as a methyl donor in the homocysteine remethylation pathway.

Although the studied group was rather small ( $n = 122$ ), it was relatively homogenous, which lowered the number of confounding variables.

## Conclusions

In elderly women, dietary patterns and plasma tHcy concentrations seems to influence fchol, TMA, and TMAO plasma concentration. Plasma TMA and fchol may be associated with BMI.

## Disclosure statement

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