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No association in maternal serum levels of TMAO and its precursors in pre-eclampsia and in non-complicated pregnancies

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ABSTRACT

Only a few studies have explored the role of microbiota-dependent metabolite trimethylamine N-oxide (TMAO) in non-complicated pregnancy and in pre-eclampsia (PE). We enrolled 139 PE and 29 healthy pregnant women in a nested case control study. We hypothesized that elevated levels of circulating TMAO and its precursors choline and glycine betaine in the late second or in third trimester might contribute to the PE and are associated with the onset of the disease and clinical features such as elevated blood pressure. The association with a few available lifestyle factors (use of fish and physical activity) was also evaluated. In contrast with the previous findings, there was no difference in TMAO concentration between PE and healthy women. In addition, TMAO concentration was not associated with any of the PE related clinical features, angiogenic or inflammatory markers. In future, it is crucial to obtain longitudinal data on TMAO in both non-complicated and in PE pregnancies before we could have more detailed understanding of TMAO.

1. Introduction

Pre-eclampsia (PE), classically defined as a new-onset hypertension and proteinuria, is a vascular disorder of the second half of pregnancy. Both hypertension and proteinuria implicate the endothelium as the target of the disease [1]. Hypertension is characterized by peripheral vasoconstriction and decreased arterial compliance. Glomerular endotheliosis, in which the glomerular endothelial cells are swollen with loss of fenestration, is considered the hallmark of PE [2].

Delivery of the placenta cures PE, yet affected women continue to have an elevated risk of premature cardiovascular diseases (CVDs) many years postpartum [3,4]. The association between PE and premature CVD is not completely understood at molecular level.

Trimethylamine N-oxide (TMAO) is a biologically active metabolite generated by the metabolism of gut microbiota from dietary precursors (choline, phosphatidylcholine, glycine betaine and L-carnitine) (Fig. 1). TMAO has gained much attention recently because of its potential adverse effects on atherosclerosis and CVD progression [5–7], and contribution to heart disease [8]. Evidence is mainly derived from observational studies although a mechanistic role for TMAO in CVD pathogenesis is also supported by animal model studies [5]. Manipulation of TMAO level modulates atherosclerosis and related processes [5,9] including endothelial dysfunction and vascular inflammation [10]. A systematic review and meta-analysis demonstrated a positive dose-dependent association between circulating TMAO concentrations and higher prevalence of hypertension [11]. However, in some clinical studies no association between TMAO level and CVD has been found [12,13]. More studies enrolling subjects with different lifestyle,

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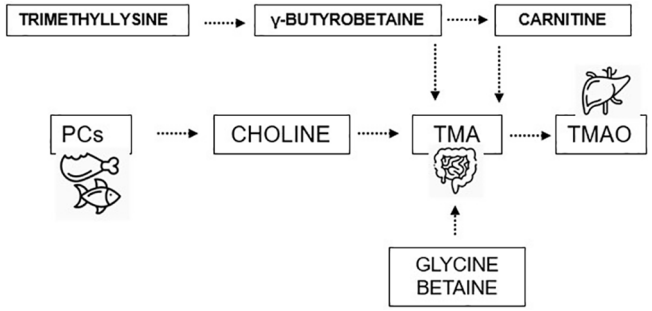

Fig. 1. Trimethylamine N-oxide (TMAO) biogenesis. TMA = trimethylamine, PC = phosphatidylcholine.

ethnicity, age, sex and underlying metabolic conditions are needed to better delineate TMAO metabolism and whether TMAO is detrimental for CVD.

The gut microbiota can metabolize above-mentioned dietary precursors to form TMA, which can be catalyzed via flavin-containing monooxygenase 3 (FMO3) to produce TMAO in the liver (Fig. 1). These precursors are abundant in red meat and in some other products that are considered to be atherogenic [5,9]. In addition to the TMAO precursors carnitine and choline, fish is a natural source of free TMAO. After a fish meal plasma TMAO levels raise rapidly even to 40-fold [14]. A very recent study [15] highlight that further studies are needed to understand the relationship between plasma levels of TMAO due to fish consumption and increased risk of CVD.

Only a few studies have explored the role of TMAO during pregnancy. An interesting recent study on mice revealed that the maternal microbiome regulates numerous small molecules including TMAO in the maternal serum and fetal neurodevelopment [16]. The data on the association between TMAO and risk of PE is also limited. In a study by Chen et al. [17], a rat model of PE showed that increased circulating TMAO promotes vascular inflammation and oxidative stress, contributing to endothelial dysfunction and hypertension in pregnancy. Furthermore, Wen et al. [18] have demonstrated that TMAO concentrations of third trimester are associated with higher risk of PE and correlate with increased systemic inflammation and endothelial dysfunction. Huang et al. [19] reported that the maternal plasma TMAO would alter over the course of pregnancy, but maternal plasma TMAO concentration in the second trimester are not associated with later PE. However, plasma TMAO level in PE, early-onset PE and PE with severe features was increased at the time of delivery when compared with non-complicated pregnancies.

A typical feature in the pathogenesis of PE is placental secretion of soluble fms-like tyrosine kinase-1 (sFlt-1), an anti-angiogenic factor, into the maternal circulation. It causes systemic endothelial injury and induces maternal multi-organ dysfunction [20]. Chang et al. [21] demonstrated that TMAO promoted sFlt-1 production in placental trophoblasts and villous explants.

We have previously reported elevated levels of carnitine precursors (trimethyllysine and gamma-butyrobetaine) in the cord plasma of newborns of PE pregnancies [22]. Currently, the knowledge of the role of carnitine precursors in different diseases is sparse.

To further understand the role of TMAO in pregnancy and in PE, we enrolled 168 pregnant women in the late second/third trimester in this nested case control study. We hypothesized that elevated levels of circulating TMAO and its precursors, choline and glycine betaine might contribute to the PE and be associated with the onset of the disease and clinical variables (blood pressure, proteinuria, high-sensitivity C-reactive protein (hs-CRP), angiogenic markers including antiangiogenic sFlt-1). Furthermore, the association with a few available lifestyle factors (use of fish and physical activity) was also evaluated.

2. Methods

2.1. Study cohort

The FINNPEC is a cross-sectional case-control multicentre study with a nationwide clinical and DNA database on PE and non-PE women, including their partners and newborns. Data of the prospective arm was assembled in Finland between 2008 and 2011. Details of the study design, methods and procedures have been described elsewhere [23]. All participants provided written informed consent, and the FINNPEC study protocol was approved by the coordinating Ethics Committee of the Hospital District of Helsinki and Uusimaa.

PE was defined as hypertension and proteinuria occurring after 20 weeks gestation according to the American College of Obstetricians and Gynecologists (ACOG) 2002 criteria [24]. PE was defined as early onset when delivery occurred before 34 + 0 weeks of gestation and late onset when at 34 + 0 weeks of gestation or later. Serum samples were collected from a subcohort from the Hospital District of Helsinki and Uusimaa. For the current metabolite analyses, we focused on a prospective arm of the study and on a subset of women of whom late second trimester and third trimester (range 24–42 weeks of gestation) serum samples were available (n = 139 for PE women and n = 29 for the non-PE controls, Table 1). All women were non-smoking. In addition, pre-existing diseases (chronic hypertension, pregestational diabetes) and

Table 1
Maternal and fetal characteristics in pre-eclamptic (PE) and control groups. Chronic hypertension was defined as systolic blood pressure ≥ 140 mm Hg and/or diastolic blood pressure ≥ 90 mm Hg detected before 20 weeks of gestation. Gestational hypertension was defined as blood pressure ≥ 140/90 without proteinuria. SD = birth weight and height converted to standard deviation scores; SGA = small-for-gestational age.

	PE	Control	*p*
n	139	29	
Gestational week at serum sampling	36 ± 4 (mean ± SD)	39 ± 2	**<0.001**[a]
Age at delivery, year	31.8 ± 5.1	32.0 ± 5.0	0.815
Nulliparous (%)	105 (75.5%)	13 (44.8%)	**0.002**
BMI, kg/m^2 (self-reported, pre-pregnancy)	24.7 ± 4.5	23.9 ± 3.3	0.446[a]
Systolic blood pressure at first antenatal visit, mm Hg	124 ± 14	113 ± 8	**<0.001**
Diastolic blood pressure at first antenatal visit, mm Hg	79 ± 10	70 ± 7	**<0.001**[a]
Early onset of PE(delivery ≤ 34 + 0 weeks of gestation)	28 (20.1%)	–	–
Preterm delivery (≤37 + 0 weeks of gestation	53 (38.1%)	1 (3.4%)	**<0.001**
Highest systolic blood pressure, mm Hg	167 ± 15	123± 9	**<0.001**[a]
Highest diastolic blood pressure, mm Hg	111 ± 9	80 ± 7	**<0.001**[a]
Proteinuria (maximum), g/24 h	4.4 ± 3.6	–	–
Chronic hypertension	27 (19.4%)	0 (0%)	**0.005**
Gestational diabetes mellitus	17 (12.2%)	0 (0%)	**0.046**
Pregestational diabetes mellitus	8 (5.8%)	0 (0%)	0.353
Fetal characteristics			
Birth weight, g	2654 ± 934	3600 ± 438	**<0.001**[a]
Relative birth weight, SD	−1.1 ± 1.4	0.1 ± 0.9	**<0.001**
SGA	33 (23.7%)	0 (0%)	**0.001**
Gestational weeks	36.2 ± 3.5	39.6 ± 1.5	**<0.001**[a]
Sex			
Girl	71 (51.1%)	11 (37.9%)	0.225[b]
Boy	68 (48.9%)	18 (62.1%)	

[a] = non-parametric test was used; [b] = Fisher's exact test.

gestational diabetes (GDM) were exclusion criteria for controls. All PE women were already diagnosed at recruitment and the late second trimester/third trimester serum samples were drawn at this timepoint in study hospitals before the delivery. The serum was stored at −80 °C.

The participants were asked to fill a detailed questionnaire on background information including data on current fish consumption and physical activity. Data were available from 145 women.

2.2. LC–MS metabolite profiling of serum

A non-targeted LC–MS metabolite profiling of maternal serum was performed earlier at Metabolomics Center at Biocenter Kuopio (University of Eastern Finland). The detailed protocol has been published earlier [22,25]. Of the identified metabolites, we utilized in the current study the third trimester levels of trimethyllysine, carnitine, choline, glycine betaine and TMAO, alongside the targeted quantitative analyses (see below).

2.3. Quantitative analysis of plasma TMAO, choline and glycine betaine

TMAO, choline and glycine betaine plasma concentrations were analyzed at Swedish Metabolomics Centre, Umeå, Sweden. 600 μL of extraction buffer (90/10 v/v methanol:water) including internal standards (TMAO- D9 (0.117 ng/μL), Choline-D9 (0.700 ng/μL) and Betaine-D9 (0.350 ng/μL) were added to 100 μL of sample material. The sample was shaken at 30 Hz for 2 min in a mixer mill and proteins were precipitated at −20 °C for 2 h. The sample was centrifuged at +4 °C, 14 000 rpm, for 10 min. To cover the broad concentration span, a vial was prepared from each sample for the TMAO panel (TMAO, choline, glycine betaine). For the TMAO panel 20 μL of the metabolite extract were transferred to a micro vial evaporated to dryness. Sets of micro vials were stored at −80 °C until analysis. Before analysis, the TMAO sample was re-suspended in 50 + 50 μL methanol and water (containing 0.4 ng/μL Proline 13C5).

Targeted liquid chromatography-tandem mass spectrometry measurements of TMAO, choline and glycine betaine were performed on a 1290 Infinity system from Agilent Technologies (Waldbronn, Germany), with an Agilent 6490 Triple quadrupole mass spectrometer for MRM-detection. The chromatography and mass spectrometry settings were the same between the two different analysis panels: 1.7 μm, 2.1 mm × 50 mm Acquity UPLC Amide column in combination with a 1.7 μm, 2.1 mm × 5 mm VanGuard precolumn (Waters Corporation, Milford, MA, USA), the column oven temperature was 85 °C. The gradient elution buffers were A (H2O, 10 mM ammonia formate) and B (90/10 acetonitrile/H2O, 10 mM ammonia formate). The initial condition was 15% A with a flow rate of 0.25 ml min −1, held for 0.1 min; A was linearly increased to 42% for 2 min and thereafter linearly increased to 90% for 0.9 min. The flow was linearly increased to 0.8 ml min-1 for 0.5 min and held for 1 min, thereafter A was decreased linearly to 15% and the flow increased to 1.6 ml min-1 for 0.5 min; these conditions were held for 0.9 min before returning to the initial conditions over 0.4 min. 1 μL of the re-suspended sample was injected for the TMAO panel and 1 μL of the extract was injected for the MNA panel.

The compounds were detected with an Agilent 6490 Triple quadrupole mass spectrometer equipped with a jet stream electrospray source operating in positive ion mode. The capillary voltage was set at 4 kV. The jet-stream gas temperature was 200 °C with a gas flow of 14 L min-1, sheath gas temperature of 325 °C, and sheath gas flow of 12 L min-1. The nebulizer pressure was set to 20 psi. The iFunnel parameters were set to 150 V and 60 V for high pressure RF and low pressure RF respectively. Dwell time was set to 0.05 s, the fragmentor was set to 380 V and the cell accelerator voltage to 7 V.

2.4. Statistical analyses

Statistical tests were performed with IBM SPSS Statistics version 26. The normality of variable distributions was verified with the Kolmogorov–Smirnov test. Logarithmic transformation was used when appropriate. For the continuous variables, comparisons between groups were analyzed with general linear model univariate ANOVA. For the categorical variables, the comparisons were performed with the Fisher's exact test. For the correlations, Spearman's correlation coefficients (r) are reported.

3. Results

Maternal and fetal characteristics are presented in Table 1. There were no differences in maternal age or body mass index (BMI) between the PE women and controls. PE women had elevated systolic and diastolic blood pressure and proteinuria according to the diagnostic criteria for PE. The proportion of nulliparous women was higher in the PE group as compared with controls. PE women suffered more from chronic hypertension and GDM. The newborns from PE pregnancies were born earlier and had smaller absolute and relative birth weight (Table 1). There was no difference in the sex distribution of the newborns.

3.1. TMAO, choline and glycine betaine concentrations

PE women had higher late second/third trimester concentrations of choline as compared with controls (Table 2). There was no difference in TMAO or glycine betaine concentrations between PE women and controls. There were no differences in TMAO, choline or glycine betaine concentrations in women with early- or late-onset PE (Table 2). Furthermore, there were no differences in these metabolites between PE women with or without chronic hypertension and/or GDM (Table 2).

When all women with or without GDM were analyzed separately, women with GDM had a trend for elevated levels of TMAO (0.25 ± 0.24 ng/μl) when compared to women without GDM (0.18 ± 0.13 ng/μl, $p = 0.117$).

3.2. TMAO correlations

TMAO concentration did not correlate with any of the PE related clinical variables (Table 3). In addition, there were no correlations between TMAO and angiogenic markers or inflammatory hs-CRP.

TMAO correlated positively in all women with late second or third trimester levels of trimethyllysine, choline and glycine betaine (Table 3). Choline and glycine betaine concentrations were available from two separate assays and the significant associations were observed only with targeted assays. Furthermore, TMAO data was available from both LC–MS metabolite profiling analysis and targeted, quantitative assay, and there was strong, highly significant positive correlation between the data obtained for TMAO from these assays (Table 3).

There was a trend for an association of plasma TMAO concentration with fish consumption (Fig. 2). The highest TMAO concentrations were observed for women who used fish two to four times per week.

The association of physical activity with plasma TMAO concentration is illustrated in Fig. 3. The lowest TMAO concentrations were observed in women who exercised 4–7 times per week.

4. Discussion

In the current study, there were no differences in TMAO or its precursor glycine betaine between PE women and controls. Furthermore, TMAO concentration was not associated with any of the PE related clinical variables, angiogenic markers or inflammatory hs-CRP concentration.

PE and hypertensive pregnancy disorders affect 3–8% and ≥ 10%, respectively, of pregnancies. The impact of these disorders is not limited to pregnancy, but they are also associated with increased risk of premature CVDs in later life. Meta-analyses indicate approximately a twofold increase in cardiovascular risk and death in women with a

Table 2
Concentrations of trimethylamine N-oxide (TMAO), choline and glycine betaine (mean ± SD) in pre-eclamptic (PE) and control women; in PE women with and without chronic hypertension and in PE women with and without gestational diabetes.

	PE all (n = 139)	Control all (n = 29)	*p*	PE early onset* (n = 28)	PE late onset (n = 111)	*p*	PE with chronic hypertension (n = 27)	PE without chronic hypertension (n = 112)	*p*	PE with gestational diabetes (n = 17)	PE without gestational diabetes (n = 122)	*p*
TMAO (ng/µl)	0.188 ± 0.156	0.175 ± 0121	0.777[a]	0.171 ± 0.125	0.192 ± 0.163	0.699	0.221 ± 0.154	0.180 ± 0.155	0.085	0.250 ± 0.243	0.179 ± 0.139	0.127
Choline (ng/µl)	1.960 ± 0.530	1.733 ± 0.391	**0.011**	1.901 ± 0.607	1.974 ± 0.510	0.518	1.976 ± 0.510	1.955 ± 0.391	0.854	1.831 ± 0.400	1.978 ± 0.544	0.288
Glycine betaine (ng/µl)	1.087 ± 0.377	1.066 ± 0.278	0.852[b]	1.103 ± 0.404	1.083 ± 0.372	0.477	1.021 ± 0.251	1.103 ± 0.400	0.434	1.004 ± 0.274	1.099 ± 0.389	0.479

*based on delivery, early ≤ 34 weeks of gestation.
[a]ln-transformed values used.
[b]non-parametric test.

Table 3
Associations of late second/third trimester trimethylamine N-oxide (TMAO) concentration with maternal and perinatal characteristics in all/PE/control subjects. AU = arbitrary unit.

Clinical parameters		r[a]	*p*	Biochemical parameters		r[a]	*p*	Metabolites		r[a]	*p*
Age of mother	All	0.10	0.24	*sFlt-1 (pg/ml), I trimester*	All	0.03	0.80	TMAO (AU), *II/III trimester*	All	**0.92**	**<0.01**
	PE	0.09	0.38		PE	0.09	0.45		PE	**0.91**	**<0.01**
	Control	0.11	0.57		Control	−0.23	0.35		Control	**0.93**	**<0.01**
BMI	All	−0.01	0.91	*sFlt-1 (pg/ml), II/III trimester*	All	0.09	0.27	Carnitine (AU), *II/III trimester*	All	0.21	0.09
	PE	−0.04	0.64		PE	0.11	0.22		PE	0.15	0.29
	Control	0.13	0.51		Control	0.01	0.97		Control	0.60	**0.03**
Parity	All	0.07	0.40	PlGF (pg/ml), I trimester	All	0.07	0.52	Choline (AU), *II/III trimester*	All	0.02	0.86
	PE	0.02	0.78		PE	0.11	0.38		PE	−0.04	0.80
	Control	0.30	0.12		Control	0.10	0.69		Control	0.28	0.33
Systolic blood pressure at first antenatal visit (mmHg)	All	0.08	0.31	PlGF (pg/ml), *II/III trimester*	All	−0.07	0.42	Choline (ng/µl), *II/III trimester*	All	**0.19**	**0.01**
	PE	0.12	0.17		PE	−0.05	0.61		PE	−0.04	0.78
	Control	−0.09	0.66		Control	−0.18	0.39		Control	0.28	0.33
Diastolic blood pressure at first antenatal visit (mmHg)	All	0.06	0.47	*Endoglin (ng/ml), I trimester*	All	−0.05	0.63	Glycine betaine (AU), *II/III trimester*	All	0.10	0.41
	PE	0.11	0.21		PE	0.02	0.90		PE	0.11	0.43
	Control	−0.26	0.17		Control	−0.34	0.17		Control	−0.04	0.91
*Highest systolic blood pressure, mmHg***	All	0.11	0.17	*Endoglin (ng/ml), II/III trimester*	All	0.02	0.80	Glycine betaine (ng/µl), *II/III trimester*	All	**0.18**	**0.02**
	PE	0.13	0.13		PE	0.02	0.86		PE	0.18	0.19
	Control	−0.28	0.15		Control	0.01	0.95		Control	0.09	0.76
Highest diastolic blood pressure, mmHg	All	0.01	0.87	*hs-CRP (mg/l), I trimester*	All	−0.13	0.32	Trimethyllysine (AU), *II/III trimester*	All	**0.49**	**<0.01**
	PE	−0.01	0.96		PE	−0.21	0.41		PE	**0.45**	**<0.01**
	Control	−0.24	0.22		Control	0.10	0.75		Control	0.50	0.07
Proteinuria (maximum), g/24 h	All	−0.07	0.42	*hs-CRP (mg/l), II/III trimester*	All	0.01	0.99				
	PE	−0.07	0.38		PE	−0.01	0.99				
	Control	–	–		Control	0.01	0.95				
Birth weight, g	All	−0.02	0.79								
	PE	−0.01	0.95								
	Control	−0.02	0.92								
Relative birth weight, SD	All	−0.06	0.45								
	PE	−0.06	0.46								
	Control	−0.03	0.90								

**When highest diastolic value recorded.
[a]Spearman's rank correlation coefficient.

history of PE [26]. The association between PE and premature CVD is incompletely understood. The dose–response relationship with the severity of a hypertensive pregnancy disorder and future CVD suggests that the differences in long-term CVD risk may be dependent on variation in the underlying maternal CVD risk profiles [27]. Elevated plasma TMAO levels and increased risk for CVD observed in humans and in several experimental studies suggest a possible involvement of TMAO in the etiology of CVD. Although it is still elusive whether TMAO by itself is a proatherogenic compound or just a biomarker of CVD [28]. To our knowledge, however, only few studies have examined TMAO in PE women [18,19,29]. Furthermore, the role of TMAO and its precursors are not extensively studied in noncomplicated pregnancy either.

The findings of this study are in contrast with the findings by Wen et al. [18] and Wang et al. [29] who showed higher serum TMAO levels in PE women compared to women with normal pregnancy. In addition, TMAO was associated with the severity of PE and TMAO levels were also positively correlated with systolic blood pressure, urinary protein levels, as well as biomarkers of inflammation/endothelial dysfunction in women with PE [18,29]. We were not able to confirm any of these associations. The reasons for the inconsistent and contradictory findings remain to be elucidated. Both studies utilized samples from late second/third trimester. Wen et al. [18] utilized serum and we used plasma samples. However, it is unlikely that a difference in sample type could explain contradictory findings. In addition, there was no difference in TMAO between PE women with or without GDM. However, when all women were analyzed together, women with GDM showed a trend for

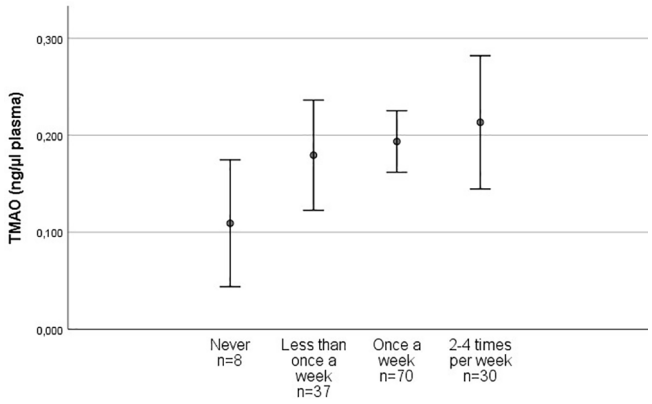

Fig. 2. Mean (95% CI) concentrations of trimethylamine N-oxide (TMAO) (ng (µl) according to use of fish, $p = 0.162$).

elevated levels of TMAO. This is in line with a recent study conducted in Chinese pregnant women which showed that TMAO in the early pregnancy is predictive of GDM [30].

In our study, PE women had higher plasma choline concentrations compared to women without PE. Accordingly, we have previously shown the rise in cord plasma choline concentrations in PE pregnancies [22]. The rise in maternal choline concentrations in PE might reflect increased demand of methyl donors and the enhanced mobilization of maternal hepatic choline stores [31]. In addition to choline, trimethyllysine and glycine betaine are also major nutrient precursors for gut microbiota-dependent generation of TMAO and we found TMAO to be correlated positively in all women. Particularly strong association was observed with TMAO and trimethyllysine. Interestingly, this intermediate in carnitine biosynthesis has been shown to be even more predictive of cardiovascular mortality than TMAO [12].

Furthermore, TMAO concentration was available from both assays. There was strong positive correlation between TMAO level measured with both non-targeted and targeted LC–MS methods. This validates the results from the non-targeted analysis and indicates that the results from the non-targeted and targeted LC–MS analyses are highly comparable in this class of compounds.

Fish is a natural source of TMAO. It has been speculated that eating more fish would lead to high TMAO levels and increased risk of CVD. However, fish consumption has mainly been shown to be cardioprotective [32]. This might be explained by the observations showing that TMAO itself can be absorbed without undergoing any transformation by gut microbiota [14]. We also observed a clear trend for fish consumption to be associated with elevated plasma TMAO concentration. Thus, it may serve as a biomarker of fish intake also for pregnant women. However, further studies with more comprehensive dietary intake information are needed.

Exercise has been shown to alter gut microbiome distribution and diversity [33]. We observed the lowest TMAO concentrations in women who reported to exercise 4–7 times per week. This is an interesting finding and worth exploring further not only in pregnant women but also in general population. Previous studies examining the effects of physical activity on TMAO are scarce. To our knowledge, Erickson et al. [34] have shown that 12 weeks of supervised exercise in the eucaloric diet did not reduce TMAO levels. Furthermore, it has been shown that compositional and functional adaptions occur in the gut microbiome in response to habitual physical activity [35–37].

The strength of the present study is a carefully characterized and prospectively recruited case-control cohort with rich clinical data. However, there are several limitations. First, we had limited number of serum available and there was imbalance between PE and control women. This was due to fact that serum samples were originally available only from a subset and they have already been utilized in the previous FINNPEC studies. Diet was assessed generally with non-validated questionnaire and only limited data was available. Particularly the lack of meat intake is a drawback. For instance, an association with physical activity may be explained by dietary confounding factors. It could be speculated that there is less meat consumption in women who exercise more. Furthermore, single sampling time point could be regarded as a limitation. In order to explore alterations in maternal TMAO metabolism throughout the whole pregnancy, it would be important to obtain samples throughout the gestation, not only from late second or third trimester as in the current study. The greater concentrations of plasma TMAO in early vs. late pregnancy have been reported and this may arise from alterations in the gut microbiome across gestation [19,31]. It is also crucial to investigate TMAO metabolism in non-complicated pregnancies before we could obtain more detailed understanding of TMAO metabolism in adverse outcomes such as in PE.

In conclusion, this is one of the very first studies to investigate TMAO and its precursors in PE and non-PE women. Our study does not support previous finding that serum TMAO concentrations are increased in PE pregnancies.

5. Collaborators for the FINNPEC Core Investigator Group

Eero Kajantie (Public Health Promotion Unit, National Institute for

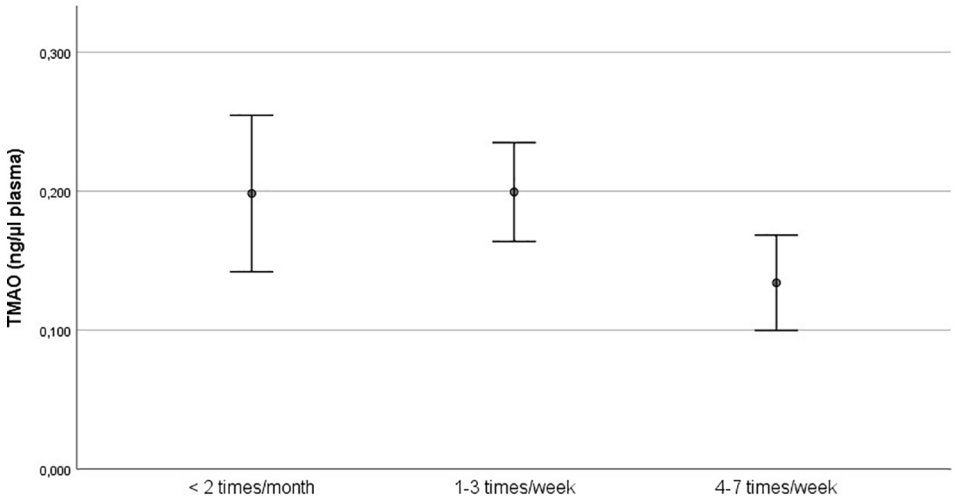

Fig. 3. Mean (95% CI) concentrations of trimethylamine N-oxide (TMAO) (ng(µl) according to frequency of physical activity, $p = 0.048$. (<2 times/month vs. 4–7 times/week, $p = 0.037$; 1–3 times/week vs. 4–7 times/week, $p = 0.020$).

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Author contributions

TJ, SH, OK, KH and HL designed the study. TJ performed the literature search. KH and OK provided the laboratory facilities and conducted the LC–MS experiments. OK and TJ performed the statistical analyses. TJ wrote the first version of the manuscript. All authors contributed to interpretation of the results. All authors critically read and edited drafts before submission. All authors read and approved the submitted version.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. KH and OK are owners of Afekta Technologies Ltd.

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