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Associations between physical activity and trimethylamine *N*-oxide in those at risk of type 2 diabetes

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ABSTRACT

Introduction Trimethylamine *N*-oxide (TMAO) has been identified as a novel gut-derived molecule that is associated with the risk of cardiometabolic diseases. However, the relationship between TMAO and physical activity is not well understood. This study prospectively investigates the association between TMAO and objectively assessed physical activity in a population at high risk of type 2 diabetes mellitus.

Research design and methods Baseline and 12-month follow-up data were used from the Walking Away from Type 2 Diabetes trial, which recruited adults at high risk of type 2 diabetes from primary care in 2009–2010. TMAO was analyzed using targeted mass spectrometry. Generalized estimating equation models with an exchangeable correlation structure were used to investigate the associations between accelerometer-assessed exposures (sedentary time, light physical activity, moderate to vigorous physical activity (MVPA)) and TMAO, adjusting for demographic, clinical and lifestyle factors in varying degrees.

Results Overall, 483 individuals had plasma samples available for the analysis of TMAO (316 (65.4%) men, 167 (34.6%) women), contributing 886 observations to the analysis. MVPA (min/day) was associated with TMAO in all models. In the fully adjusted model, each 30 min or SD difference in MVPA was associated with 0.584 µmol/L (0.070, 1.098) and 0.456 µmol/L (0.054, 0.858) lower TMAO, respectively. Sedentary time and light physical activity were not associated with TMAO in any model.

Conclusions Engagement with MVPA was associated with lower TMAO levels, suggesting a possible new mechanism underlining the inverse relationship between physical activity and cardiometabolic health.

Significance of this study

What is already known about this subject?

- Trimethylamine *N*-oxide (TMAO) is associated with the risk of cardiometabolic diseases.
- It is unknown whether physical activity, which is fundamental to cardiometabolic health, is also associated with TMAO.

What are the new findings?

- Moderate to vigorous physical activity is inversely associated with TMAO in individuals at risk of type 2 diabetes.
- This association was independent of other cardiometabolic risk factors and diet.

How might these results change the focus of research or clinical practice?

- Physical inactivity may be a risk factor for elevated TMAO levels.

INTRODUCTION

Physical activity and diet induce a myriad of physiological adaptations that can be beneficial to human cardiometabolic health, either directly or indirectly. Recent evidence has highlighted the role of the gut microbiota in cardiometabolic health through several mechanisms, such as increased production of the microbial metabolite trimethylamine *N*-oxide (TMAO). TMAO is a small, organic dietary compound that is generated by the gut following ingestion of dietary L-carnitine and phosphatidylcholine-rich foods such as red meat and eggs.[1] TMAO generation is dependent on the gut microbiota, which first metabolizes dietary choline to trimethylamine which is then converted to TMAO in the liver by enzymes of the flavin monooxygenase family.[1]

TMAO has been identified through untargeted metabolomic studies as a molecule that is present in systemic circulation and associated with the risk of atherosclerosis and cardiovascular disease development.[2 3] Recent systematic reviews and meta-analyses have confirmed a positive dose-dependent association between TMAO plasma levels and increased cardiovascular disease risk and mortality.[4–6] Several studies have also found that plasma TMAO levels are higher in people with type 2 diabetes compared with those without,[7–9] although the direction of causality has been questioned.[10]

Numerous studies have also assessed the relationship between diet, gut microbiome and TMAO levels,[11–13] with studies observing that TMAO is increased in omnivores compared with vegans or vegetarians.[1] Importantly, those with a less healthy gut microbiota profile, evidenced by a higher proportion of Firmicutes relative to Bacteroidetes and less bacterial diversity, produce more TMAO than those with a healthier profile in response to the ingestion of the same dietary stimulus.[14]

Recent findings also suggest that physical activity can positively alter gut microbiota, resulting in favorable health outcomes.[15] Having an active lifestyle may positively influence gut microbiota via several possible mechanisms including the alteration of gut bacterial composition and diversity.[16 17] Indeed, the benefits of physical activity on the gut microbial communities appear to be independent of diet.[18] Therefore, physical activity may promote a healthier gut microbiome profile that in turn produces less harmful bioactive metabolites including TMAO, particularly in older or obese individuals who are more likely to have an unhealthy gut microbiota profile.[19 20]

TMAO levels are also influenced by other factors. For example, circulating levels of TMAO are highly dependent on renal function,[21] with research showing that as estimated glomerular filtration rate (eGFR) decreases, TMAO levels rise proportionally in patients with chronic kidney disease.[22] As higher physical activity has been associated with higher eGFR levels in some studies,[23 24] it is possible that physical activity also affects TMAO through renal function.

Although the interaction between physical activity, gut microbiota, and cardiovascular disease has gained recent interest,[16 25] the link between physical activity and TMAO is currently not well documented. The aim of this study is to provide new evidence on the independent prospective association between TMAO and physical activity in a population at high risk of type 2 diabetes.

METHODS

Study design and participants

This study uses prospective observational data generated from the Walking Away from Type 2 Diabetes trial in those identified with a high risk of type 2 diabetes recruited form a primary healthcare setting. The design and results of the Walking Away from Type 2 Diabetes trial have been described in detail previously.[26 27] In brief, individuals were recruited from 10 general practices in Leicestershire, UK, during 2009–2010. Individuals were recruited based on having a high risk of type 2 diabetes defined as impaired glucose tolerance (2-hour glucose ≥7.8 and <11.1 mmol/L), and/or impaired fasting glycemia (fasting glucose ≥6.1 and <7.0 mmol/L) or undiagnosed type 2 diabetes, using the Leicester Practice Risk Score.[26 28] The score calculates risk based on six variables (age, sex, ethnicity, body mass index (BMI), family history of the disease, and antihypertensive drug usage). The study excluded people with established type 2 diabetes, type 2 diabetes diagnosed at baseline, those currently taking steroids, and those unable to take part in any walking activity. General practices were randomized to receive a standardized information leaflet (control) or the Walking Away intervention. The Walking Away intervention consisted of an initial 3-hour group-based structured educational program, delivered to small groups by trained educators and annual group-based refresher education sessions. The primary aim of the intervention was to increase physical activity through walking activity.[26 27]

Anthropometric data

Body mass (Tanita TBE 611; Tanita, West Drayton, UK) was measured to the nearest 0.1 kg with height measured to the nearest 0.5 cm. BMI was calculated by dividing mass to squared height (kg/m^2). Arterial blood pressure was measured in the sitting position after resting for 5 min (Omron Healthcare, Henfield, UK); three measurements were obtained and the average of the last two measurements was used. Information on age, current smoking status, medication history and ethnicity was obtained by interview.

Biochemical data

Fasted blood samples were collected at a baseline visit and then 12 months later. Lipid profile (triglycerides, high-density lipoprotein (HDL) cholesterol and total cholesterol), creatinine and HbA1c were analyzed at the clinical laboratory within Leicester Royal Infirmary hospital according to standardized quality-controlled procedures. Creatinine was used to calculate eGFR using the Modification of Diet in Renal Disease equation which was categorized as normal kidney function (eGFR >90), mildly impaired kidney function (eGFR=60–90), and moderately impaired kidney function (eGFR <60).[29] Remaining plasma samples were quantified in duplicate for TMAO levels using stable isotope dilution (D9-TMAO (98.0% purity)) followed by ultra-performance liquid chromatography-tandem mass spectrometry analysis. This was performed on a Shimadzu Nexera X2 LC-30AD coupled with a Shimadzu 8050 triple quadrupole mass spectrometer (Shimadzu, Kyoto, Japan), using optimized conditions of a previously described method with a coefficient of variation of 3.6% for measurements throughout the study.[30 31]

Physical activity and dietary data

Physical activity was measured by an accelerometer (ActiGraph GT3X, Pensacola, Florida, USA) worn on the right anterior axillary line above the hip for 7 consecutive days during waking hours. Accelerometers were initialized with a 15 s epoch, with data reintegrated into 60 s epochs for the purposes of this analysis. Freedson cut-points were used to derive intensity thresholds, with <100 counts/min estimating time spent sedentary, 100–1951 counts/min estimating light-intensity physical activity, and ≥1952 counts/min estimating time spent in moderate to

vigorous intensity (moderate to vigorous physical activity (MVPA)).[32] At least 4 valid days of data were required for inclusion in this analysis, with a valid day consisting of at least 600 min of wear time; non-wear time was defined as more than 60 min of continuous zero count.[33] Data were processed using a commercially available software package (KineSoft V.3.3.76, KineSoft, Loughborough, UK; www.kinesoft.org).

Diet was measured using the Dietary Instrument for Nutrition Education (DINE) food frequency questionnaire at baseline and 12 months, a brief self-report tool for diet assessment, which provides a score for total fat, unsaturated fat and fiber intake.[34] Weekly servings of red meat (beef, pork or lamb) and fish were extracted to be used as covariates as they contain dietary precursors to TMAO generation.[1] Red meat and fish consumption was coded as 0 if participants answered 'None' or 'Less than 1 a week'; 1.5 if the answer was '1–2 a week'; 4 if the answer was '3–5 a week'; and 6 if the answer was '6 or more a week'.

Data inclusion

The Walking Away intervention was unsuccessful at promoting changes to sedentary time, light physical activity or MVPA at any time point.[27] Hence, for the purposes of this study, the trial cohort was pooled and data were analyzed observationally using the combined cohort. From the 808 individuals included in the Walking Away from Type 2 Diabetes trial at baseline, 500 (62%) had stored plasma samples that enabled the analysis of TMAO at both baseline and 12 months, with the remainder providing insufficient blood volume for storage. Of those with TMAO data, 483 had concurrent accelerometer data at baseline or 12-month follow-up (see figure 1).

Statistical analysis

All analyses were conducted using IBM SPSS Statistics (V.24.0). Included data were used to form an observational cohort in order to examine whether MVPA, light physical activity and sedentary time are associated with TMAO. A generalized estimating equations model with an exchangeable correlation structure was used to allow for repeated measurements at baseline and 12 months. Continuous TMAO data displayed a positive skewed distribution and were therefore analyzed using a gamma distribution with an identity link.

Three main accelerometer-derived exposures were investigated: average light physical activity, average MVPA and average sedentary time. Model 1 was unadjusted, model 2 included age, sex, ethnicity, time, smoking status, randomization group (intervention/control) and accelerometer wear time. Model 3 was additionally adjusted for kidney function, HDL cholesterol, triglycerides, BMI, systolic blood pressure, HbA1c, lipid-lowering medication (statins and fibrates) and blood pressure medication (ACE inhibitors, β blockers, α blockers, calcium channel blockers, diuretics and angiotensin II receptor blockers). Model 4 was additionally adjusted for red meat and fish intake. Regression coefficients are reported as per 30 min difference and per SD difference in behavior.

In order to assess whether reported associations were consistent across levels of glycemic control in this high risk of type 2 diabetes population, an interaction term for HbA1c as a continuous variable was added to model 3. $P<0.05$ was considered significant for main effects, and $p<0.1$ was considered significant for interactions.

Sensitivity analysis

A sensitivity analysis was carried out using model 3 and adjusted for sedentary time if MVPA was the exposure and vice versa. This allowed us to examine whether sedentary time or MVPA were independently associated with changes in TMAO plasma levels.

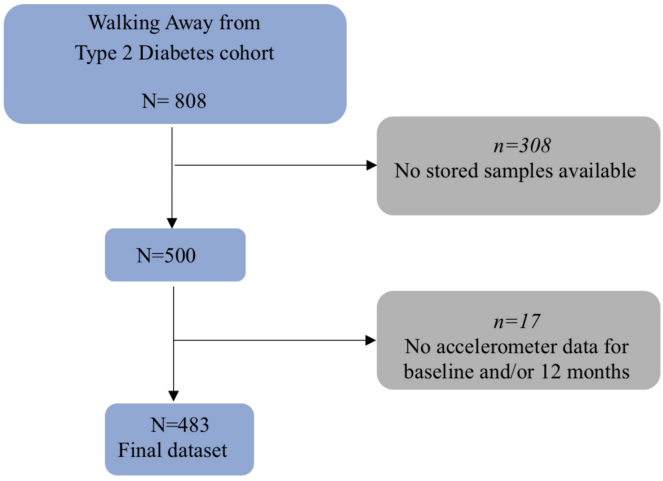


Figure 1 Flow diagram of included participants.

RESULTS

Overall, 483 individuals were included (316 (65.4%) men and 167 (34.6%) women) contributing 886 observations to the analysis (see figure 1). The baseline and follow-up characteristics of the participants included in this study are shown in table 1. The mean (SD) age and BMI of included participants were 63.5 years (7.3) and 32.2 kg/m^2 (5.5), respectively, with the majority of individuals included in this analysis being White European (437 (90.5%)). Online supplemental table S1 shows the baseline characteristics for 325 participants excluded due to insufficient stored plasma samples for TMAO analysis compared with the 483 included characteristics were largely similar across the included and excluded data sets.

Figure 2 (data shown in online supplemental table S2) reports the associations of differences in light physical activity, MVPA and sedentary time with TMAO using four different models.

Each 30 min or SD difference in MVPA per day was associated with 0.574 µmol/L (0.033, 1.116) and 0.449 µmol/L (0.026, 0.872) lower TMAO, respectively, in the unadjusted model. The association was largely unaffected with adjustment for clinical, sociodemographic

Table 1 Demographics, metabolic, anthropometric, physical activity and dietary characteristics of included participants (n=483)

Characteristics	Baseline	n	12-month follow-up	n
Age (years)	63.5±7.3	483		
Sex				
Men	316 (65.4)			
Women	167 (34.6)			
Smoking status				
Never smoked	445 (92.1)			
Current smokers	38 (7.9)			
Ethnicity				
White European	437 (90.5)			
Other	46 (9.5)			
Treatment group				
Intervention	246 (50.9)			
Control	237 (49.1)			
Kidney function		446		438
Healthy	155 (34.8)		112 (25.5)	
Mildly impaired	271 (60.8)		301 (68.4)	
Moderately impaired	20 (4.5)		25 (5.7)	
Body mass index (kg/m^2)	32.2±5.5	483	31.9±5.6	481
Systolic blood pressure (mm Hg)	143.2±18.4	483	133.1±17.2	481
HbA1c (%)	5.9 (5.6–6.1)	481	5.9 (5.7–6.1)	478
HDL cholesterol (mmol/L)	1.4 (1.2–1.6)	482	1.3 (1.2–1.6)	476
Triglycerides (mmol/L)	1.4 (0.9–1.7)	483	1.3 (1.0–1.9)	481
TMAO (µmol/L)	4.8 (3.1–7.5)	483	5.0 (3.4–8.0)	483
Physical activity variables				
Accelerometer variables				
Wear time (min/day)	860.5±85.3	446	857.2±84.9	440
Sedentary time (min/day)	542.5±102.9		550.4±103.9	440
Light-intensity physical activity (min/day)	289.6±78.3		280.2±79.9	440
Moderate to vigorous-intensity physical activity (min/day)	28.4±24.5		26.7±22.3	440
Dietary variables				
Red meat (beef, pork or lamb) (servings/week)	4.0 (1.5–4.0)	387	4.0 (1.5–4.0)	319
Fish (servings/week)	1.5 (0.0–1.5)	336	1.5 (0.0–1.5)	336

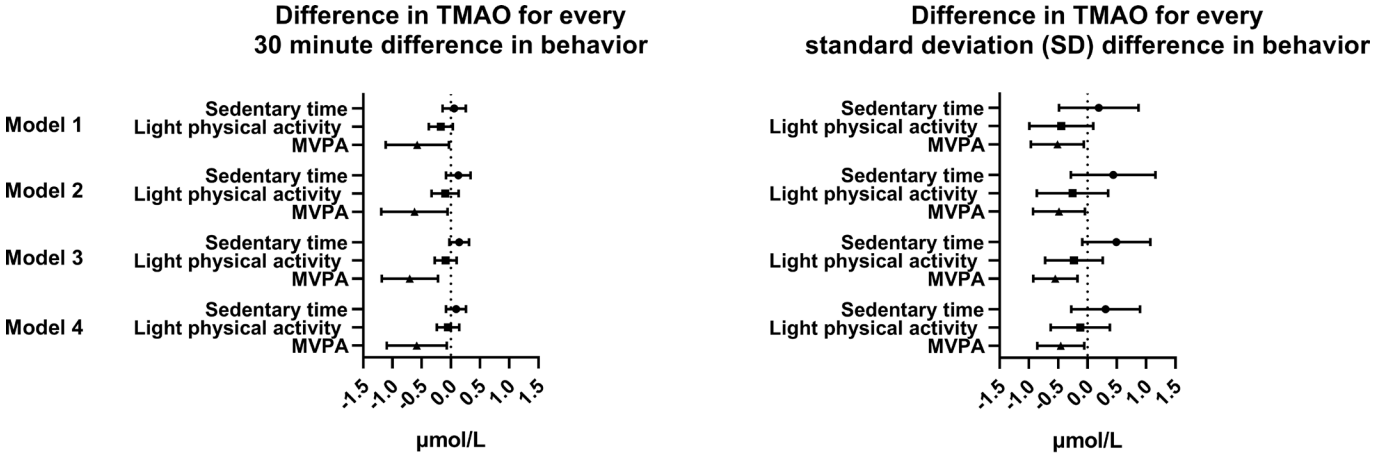
Continuous parametric results are displayed as mean±SD or number (percentage), and continuous non-parametric results are displayed as median (IQR); sedentary time=<25 counts/15 s, light-intensity activity ≥25 to <488 counts/15 s, and moderate to vigorous physical activity (MVPA) ≥488 counts/15 s.
HDL, high-density lipoprotein; TMAO, trimethylamine *N*-oxide.

or lifestyle (including dietary) factors, with each 30 min or SD difference in MVPA associated with 0.584 µmol/L (0.070, 1.098) and 0.456 µmol/L (0.054, 0.858) lower TMAO, respectively (figure 2; online supplemental table S2). Sedentary time and light-intensity physical activity were not associated with TMAO in any model (figure 2; online supplemental table S2).

Glycemic control (HbA1c) did not modify the associations for MVPA (p=0.269 for interaction) with TMAO.

Sensitivity analysis

Results for sedentary time and MVPA were unaffected when mutually adjusted (table 2), with MVPA continuing to be associated with TMAO.



Data as coefficient (95%CI)

Figure 2 Association between physical activity exposures and trimethylamine *N*-oxide (TMAO). Model 1: unadjusted. Model 2: age, sex, ethnicity, smoking status, treatment group, wear time. Model 3: model 2+kidney function, high-density lipoprotein (HDL) cholesterol, triglycerides, body mass index (BMI), systolic blood pressure, HbA1c, lipid-lowering medication, blood pressure-lowering medication. Model 4: model 3+red meat and fish. MVPA, moderate to vigorous physical activity.

DISCUSSION

TMAO, a novel gut-derived metabolite, has increasingly been implicated with an elevated risk of developing cardiometabolic diseases,[2–9] with some, but not all, research suggesting these associations are causal.[10 35] In this study, MVPA was inversely associated with TMAO plasma levels independently of age, sex, ethnicity, smoking status, kidney function, HDL cholesterol, triglycerides, BMI, systolic blood pressure, HbA1c, medication, and red meat and fish consumption. Each 30 min/day of MVPA was associated with 0.584 µmol/L lower TMAO. Sedentary time and light physical activity were not found to be associated with TMAO, suggesting intensity of movement may be important. These results highlight a potential new mechanism for the observed relationship between MVPA and cardiometabolic risk. To our knowledge, this is the first study to investigate the association between physical activity and TMAO.

It is widely established that MVPA is associated with improvements in cardiometabolic health, with previously elucidated mechanisms including pathways related to insulin resistance, lipid metabolism, and chronic low-grade inflammation.[36–38] However, traditional risk factors and mechanisms do not explain all of the association between physical activity and health outcomes.[39–41] In this study, adjustment for a wide range of clinical markers that have the potential to influence TMAO, including kidney function, did not attenuate association with MVPA suggesting alternative pathways may, at least in part, mediate reported associations. Gut microbiota have been implicated as an endocrine organ that plays an important role in the regulation of the hosts' cardiometabolic health through modulating levels of bioactive metabolites, of which TMAO is one. The amount of TMAO produced in relation to choline and betaine challenge has been associated with the profile and diversity of the gut microbiome.[1 2 42] As the profile and diversity of the gut microbiome have also been associated with MVPA, particularly in older or obese adults,[16 17 43] MVPA may help promote a healthier gut microbiota environment that is less conducive to TMAO generation, providing an important hypothesis for future research targeting the manipulation of the microbiome and its metabolites in the promotion of cardiometabolic health.

Although this is the first study to specifically focus on the association between physical activity and TMAO, others have investigated the effect of lifestyle interventions that have included a physical activity component, with equivocal findings. A small experimental study found no change in TMAO following a eucaloric diet

Table 2 Sensitivity analysis showing associations with TMAO (µmol/L) when MVPA and sedentary time were mutually adjusted

Covariate	Coefficient per 30 min (95% CI)	Coefficient per SD (95% CI)	P value	Participants (n)	Observations included (n)
MVPA	−0.561 (−1.119 to −0.004)	−0.438 (−0.003 to 0.873)	0.048	449	738
Sedentary time	0.017 (−0.168 to 0.202)	0.059 (−0.575 to 0.692)	0.933	449	738

Model mutually adjusted for MVPA and sedentary time along with age, sex, ethnicity, time, smoking status, randomization group, accelerometer wear time, kidney function, high-density lipoprotein (HDL) cholesterol, triglycerides, body mass index (BMI), systolic blood pressure, HbA1c, lipid-lowering medication, blood pressure-lowering medication, meat intake, and fish intake.
MVPA, moderate to vigorous physical activity; TMAO, trimethylamine *N*-oxide.

combined with exercise.[44] Similarly, post hoc analysis of the Tübingen Lifestyle Intervention Program study did not observe any changes in TMAO following a lifestyle intervention (targeting diet, weight loss and physical activity) in those at risk of type 2 diabetes.[45] However, when the cohort was divided into tertiles of change in TMAO, those that did decrease their TMAO following the intervention were found to have higher baseline TMAO. The average baseline TMAO (µmol/L) levels in the current study were higher than in Tübingen Lifestyle Intervention Program. Given that the effect of exercise training on the gut microbiota environment has been shown to be depended on obesity status,[43] physical activity and lifestyle may be particularly important interventions for targeting TMAO in metabolically high-risk obese individuals where TMAO is already elevated. However, given the dearth of evidence in this area, further intervention studies are needed to specifically investigate the impact of physical activity and exercise training on TMAO.

This study has important strengths and limitations. The main strength is that it provides novel evidence that TMAO may be modulated by lifestyle behaviors beyond diet. The use of objective measures of physical activity and sedentary behavior to accurately capture intensity of physical activity is also a strength. However, it is important to acknowledge some limitations. The Walking Away intervention was not successful at initiating behavior change in MVPA, light-physical activity or sedentary time, therefore the Walking Away study cohort was used to investigate observational associations with TMAO rather than the effects of behavior change. Limitations specific to observational research therefore apply, including the inability to ascribe causation and the potential for confounding by unmeasured, or residual confounding by poorly measured factors. Moreover, while the use of DINE food frequency questionnaire allows for inclusion and adjustment for the main TMAO precursors or sources (red meat and fish), it does not provide assessment of all potential precursors (ie, eggs and dairy products). Therefore, it is possible that reported results for MVPA were affected by residual or unmeasured dietary confounding. Finally, the sample was recruited on the basis of having risk factors for type 2 diabetes and may not therefore be representative of the wider population.

In conclusion, this study suggests that more time spent in MVPA may be associated with lower TMAO, independent of other cardiometabolic risk factors and diet. However, given the observational nature of this study, further research exploring the effects of changing physical activity volume and intensity on TMAO is warranted.

Perspective

Physical activity is fundamental to cardiometabolic health status, with the most investigated pathways related to insulin resistance, lipid metabolism, and chronic low-grade inflammation.[36–38] Gut microbiota and associated metabolites may also have a role to play.[16 17 43] This study provides novel evidence that TMAO, one of the most researched gut metabolites which is hypothesized to have a deleterious effect on cardiometabolic health, is inversely associated with MVPA and that this relationship was present independent of red meat consumption in individuals at risk of type 2 diabetes mellitus. These findings suggest a potential novel mechanism underpinning the inverse relationship between physical activity and cardiometabolic disease, highlighting that physical inactivity may be a risk factor for elevated TMAO levels.

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Competing interests None declared.

Patient consent for publication Not required.

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