Marked elevation in plasma trimethylamine-N-oxide (TMAO) in patients with mitochondrial disorders treated with oral L-carnitine

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ABSTRACT

Oral supplementation with L-carnitine is a common therapeutic modality for mitochondrial disorders despite limited evidence of efficacy. Recently, a number of studies have demonstrated that a gut microbiota-dependent metabolite of L-carnitine, trimethylamine oxide (TMAO), is an independent and dose-dependent risk factor for cardiovascular disease (CVD). Given the limited data demonstrating efficacy with oral L-carnitine therapy and the newly raised questions of potential harm, we assessed plasma TMAO levels in patients with mitochondrial disease with and without oral L-carnitine supplementation. Nine subjects were recruited and completed the study. Eight out of 9 subjects at baseline had plasma TMAO concentrations < 97.5th percentile (< 15.5 μM). One subject with stage 3 renal disease, had marked elevation in plasma TMAO (pre 33.98 μM versus post 101.6 μM). Following at least 3 months of L-carnitine supplementation (1000 mg per day), plasma TMAO levels were markedly increased in 7 out of 9 subjects; overall, plasma TMAO significantly increased 11.8-fold (p < 0.001) from a baseline median level of 3.54 μM (interquartile range (IQR) 2.55–8.72) to 43.26 μM (IQR 23.99–56.04) post supplementation. The results of this study demonstrate that chronic oral L-carnitine supplementation markedly increases plasma TMAO levels in subjects with mitochondrial disorders. Further studies to evaluate both the efficacy and long term safety of oral L-carnitine supplementation for the treatment of mitochondrial disorders are warranted.

1. Introduction

A variety of vitamins, cofactors and supplements are commonly used in the treatment of mitochondrial disorders. L-carnitine has been proposed as a treatment modality on the basis of its role in translocation of long chain fatty acids into the mitochondria for beta-oxidation and ATP production [1]. Studies have demonstrated that increasing muscle carnitine can enhance fatty acid oxidation while sparing glycogen [2] in healthy subjects and prevent fat gain during low intensity exercise [3,4]. However, other studies have failed to demonstrate an effect on substrate utilization [5,6]. Variance in study protocols may at least partially account for conflicting results as it has been suggested that co-ingestion of oral L-carnitine with carbohydrate is critical to increase muscle carnitine stores and protein can blunt the effect [7]. However, in a recent study of healthy subjects, no enhancement of exercise performance was demonstrated, despite documented increase in skeletal muscle carnitine, during a 24 week trial of high intensity interval training [8].

Studies of oral L-carnitine supplementation in patients with mitochondrial disorders are limited. A study of 12 patients with mitochondrial myopathy and chronic progressive external ophthalmoplegia, found a modest increase in oxygen consumption and exercise tolerance during a standardized exercise test, after an 8 week trial of oral L-carnitine [9]. A limitation of this study was that there was no measurement of muscle carnitine. No long-term placebo-controlled studies have been completed to test the safety of oral L-carnitine therapy. Based on limited evidence to support efficacy, a recent consensus statement from the Mitochondrial Medicine Society, did not include oral L-carnitine as a therapeutic modality [10,11]. Yet in practice, and despite the recently published consensus statement, many mitochondrial patients remain on oral L-carnitine supplementation, and the United Mitochondrial disease foundation, a patient association, lists oral L-carnitine as a “first tier” supplement (https://www.umdf.org/what-is-mitochondrial-disease/treatments-therapies/).

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l-carnitine, an abundant nutrient in red meat, is metabolized by intestinal bacteria to form a volatile metabolite, trimethylamine (TMA), which is converted to trimethylamine-N-oxide (TMAO) by the hepatic enzyme, flavin monoxygenase 3 (FMO3) (Fig. 1). A potentially harmful effect of TMAO on cardiovascular health has been a focus of intense research in recent years. In 2011, metabolomics studies identified an association between TMAO and cardiovascular disease (CVD) in humans, and mechanistic studies indicated TMAO can accelerate atherosclerosis in animal models of disease [12]. Further in vivo studies established that dietary l-carnitine can serve as an alternative nutrient precursor for the generation of both TMA and TMAO in both humans and mice alike, and animal model studies showed that dietary supplementation with l-carnitine can accelerate atherosclerosis in atherosclerosis prone apolipoprotein E-/− mice [13]. Moreover, large scale clinical studies revealed that plasma levels of carnitine are dose dependently associated with incident risk for major adverse cardiovascular events (myocardial infarction (MI), stroke and death), and that the adverse risk associated with plasma carnitine levels is only observed amongst those who concomitantly have elevated TMAO levels [13]. The relationship between plasma TMAO and CVD was further studied in patients (n = 4007 subjects) undergoing elective coronary angiography. Compared to participants with TMAO in the lowest quartile, those in the highest quartile (> 6.2 μM) had a significantly increased risk of incident CVD events such as MI, stroke or death independent of other cardiovascular risk factors [14]. Multiple studies have since confirmed an association between systemic TMAO levels and incident CVD event risk in various patient populations. Moreover, several meta-analyses have recently been published that systematically review the TMAO literature, all of which conclude that plasma TMAO is an independent and dose-dependent risk factor for CVD and mortality risks [15–17].

A mechanistic role for TMAO in development of CVD has been supported by multiple additional studies. TMAO in animal model studies has been shown to promote aortic endothelial cell activation, and up regulation of inflammatory gene signatures [18]. Modulation of TMAO levels by suppression of FMO3 levels using antisense oligonucleotide targeted approaches has been shown to inhibit atherosclerosis in several animal model studies, including hypercholesterolemic LDL-R-/− models [19], and to impact tissue cholesterol homeostasis [18,20]. TMAO has also been linked to changes in visceral adipose tissue phenotype and metabolism [21]. TMAO has also been shown to directly interact with platelets, rapidly rendering them more responsive to agonists like ADP, thrombin and collagen, leading to changes in intracellular calcium signaling, hyper-responsiveness, and enhanced thrombosis potential in vivo [22]. Moreover, TMAO levels have been shown to dose-dependently be associated with incident thrombotic event risks in patients [22,23]. In recent human clinical intervention studies, supplemental choline provided to both omnivore and vegan/vegetarian alike was shown to result in elevation in plasma TMAO levels, and heightened platelet aggregation responses in subjects, even in the presence of aspirin therapy [24]. Notably, in recent studies, use of a small molecule inhibitor that attenuates gut microbial production of TMA, and thus systemic TMAO levels, was shown to significantly reduce diet dependent atherosclerosis development in animal models [25].

Thus, a growing body of evidence is accruing indicating that the gut microbe-generated metabolite, TMAO, is linked to CVD and thrombosis risks. Given the limited evidence of benefit from oral l-carnitine supplementation in subjects with mitochondrial disorders, and the mounting evidence that TMAO both serves as a risk factor for CVD and adverse event risks in subjects, we sought to determine the degree to which oral l-carnitine supplementation increases TMAO levels in patients with mitochondrial disease.

2. Materials and methods

The Adult Metabolic clinic is the referral centre for patients suspected of mitochondrial disorders in British Columbia. Patients coded as having a highly probable or definite diagnosis of mitochondrial disease and undergoing standard treatment, were invited to participate in the study. Patient characteristics and diagnoses are summarized in Table 1. A diagnosis of mitochondrial disease was based on suggestive clinical features and either a pathogenic, or likely pathogenic nuclear gene or mtDNA mutation(s) as defined by ACMG criteria [26]. Ten patients were recruited into the study.

Diet history: Patients completed a detailed diet history over the telephone with a Metabolic Dietitian. Each diet history was analyzed for carnitine content using the on-line diet analysis software by Carnipure TM. An average daily intake of carnitine was established for each patient.

| Table 1 | Patient characteristics. |
|---------|-------------------------|
| Case #  | Age (years) | Sex (M/F) | Diagnosis | Creatinine (Cr) uM/eGFR, (ml/min/1.73m²) | Diet |
| 1       | 48          | M          | Predominant single mtDNA deletion 14 kb | Cr 43 eGFR> > 120 | Vegetarian |
| 2       | 42          | F          | OPA c.2242C > T p.Arg748, | No testing | Omnivore |
| 3       | 74          | F          | Multiple mtDNA deletions | Cr 124 eGFR 37 | Omnivore |
| 4       | 63          | F          | Multiple mtDNA deletions | Cr 46 eGFR 103 | Omnivore |
| 5       | 66          | F          | m.8344 A > G (MERFF) | Cr 62 eGFR 91 | Omnivore |
| 6       | 53          | F          | m.3243 A > G (MELAS) | Cr 51 eGFR 62 | Omnivore |
| 7       | 59          | M          | m.3243 A > G (MELAS) | Cr 64 eGFR 103 | Omnivore |
| 8       | 64          | F          | m.3243 A > G (MELAS) | No testing | Omnivore |
| 9       | 74          | M          | TWINKLE c.1105 T > C p.Ser369Pro | Cr 79 eGFR 84 | Omnivore |
| 10      | 38          | F          | TWINKLE c.1105 T > C p.Ser369Pro | Cr 66 eGFR 103 | Omnivore |

Eight out of 10 patients received 1000 mg oral l-carnitine daily, either divided into 2 or 3 doses for > 1 year prior to blood collection. Patients were off carnitine for at least 3 months before collection of the 2nd “off carnitine” blood sample. Subject 6 had a baseline sample collected off carnitine, and then placed on oral l-carnitine for 3 months prior to 2nd blood collection. Subject 2 was omitted from data analysis – incorrect timing of “on carnitine” sample – collected 1 week after resuming oral l-carnitine therapy. Patient 3 noted to have significant renal impairment.

* eGFR calculated using the CKD-EPI (Chronic kidney disease epidemiology collaboration) equation. Levey et al. A New Equation to Estimate Glomerular Filtration Rate. Ann Intern Med. 2009;150:604–612.
Two blood samples were collected on each patient, on and off oral l-carnitine therapy. Nine out of 10 had been receiving oral l-carnitine (levocarnitine, 3-carboxy-2(R)-hydroxy-N,N,N-trimethyl-1-propanaminci, inner salt) 1000 mg daily, either divided in 2 or 3 doses, for >1 year, at the time of study recruitment. Blood samples were collected shortly after study recruitment and then again 3 months after discontinuing oral l-carnitine therapy. One patient was not receiving oral l-carnitine at the time of recruitment and was placed on oral l-carnitine for 3 months prior to the 2nd blood collection.

Methods: Plasma TMAO analysis was performed using stable isotope dilution tandem mass spectrometry on a Shimadzu 8050 triple quadrupole mass spectrometer, as previously described [27].

Statistical analysis: Paired t-test was used to compare the intra-individual difference in TMAO concentration on/off carnitine.

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3. Results

Patient characteristics (n = 10) are summarized in Table 1 along with plasma TMAO values on and off carnitine. Nine patients were omnivores and one patient was strictly vegetarian. One patient, subject 2, was omitted from data analysis due to incorrect timing of “on carnitine” sample.

Subjects (n = 9) at baseline had a median plasma TMAO of 3.54 μM (interquartile range (IQR) 2.55-8.72; range 1.94-33.98) and on oral l-carnitine had a median plasma TMAO of 43.26 (IQR 23.99-56.04, range1.55-101.6). The median TMAO level on oral l-carnitine therapy (33.98 versus 101.6 μM). All subjects had a significant increase in plasma TMAO while receiving oral l-carnitine except subject 1, the only vegetarian in the study.

4. Discussion

The majority of patients in this study had marked elevation of plasma TMAO while receiving oral l-carnitine therapy. Given the strong evidence that TMAO is a risk factor for CVD, this raises concern regarding the safety of long-term oral l-carnitine use. Safety studies evaluating long-term exposure to oral l-carnitine in mitochondrial patients have not been performed. Oral l-carnitine is routinely prescribed for a number of inborn errors of metabolism including organic acidemias and primary carnitine uptake deficiency to remove build-up of harmful metabolites and/or replenish depleted carnitine stores [28-30]. Survival of patients with inborn errors of metabolism well into adulthood is now common yet long-term controlled safety and efficacy studies have not been done and have been recently been recommended [31].

A recently published consensus statement on the treatment of mitochondrial disorders does not include oral l-carnitine as a recommended treatment [10,11]. Although there is a lack of evidence of efficacy, this does not mean that carnitine is ineffective. Patients currently taking oral l-carnitine may be reluctant to discontinue treatment and physicians may be reluctant to change their current prescribing practice.

Alternative delivery models of carnitine have been suggested such as cyclic administration of oral antibiotic or periodic parenteral l-carnitine supplementation to reduce TMAO levels while maintaining sufficient carnitine [31]. It has been suggested that a vegetarian diet might protect against TMAO production from oral carnitine. Studies have demonstrated striking differences in the composition of gut microbiota in vegetarians and vegans compared with omnivores. Red meat, which is rich in carnitine, affects the composition of the gut microbial community promoting growth of bacterial species that metabolize l-carnitine to TMA. Vegetarians and vegans have reduced capacity to make TMA from dietary l-carnitine and have lower plasma TMAO levels compared to omnivores [32]. Only one patient in this study was a strict vegetarian and interestingly had no increase in TMAO after three months of recommended oral l-carnitine therapy. However, exposure to oral l-carnitine in animal models has demonstrated increased TMA synthetic capacity 10 fold with a concurrent shift in gut microbial composition. Patients with inborn errors of metabolism, on low protein/meat restricted diets have marked elevation of TMAO on chronic oral l-carnitine therapy [31]. Therefore, a vegetarian diet may not protect against TMAO production with longterm use of oral l-carnitine.

The only patient in this study with marked elevation in plasma TMAO at baseline had chronic kidney disease (CKD stage 3). Plasma TMAO is cleared by the kidney and studies have demonstrated that TMAO levels are significantly elevated in CKD [33]. It has been suggested that TMAO contributes to cardiovascular disease in the context of CKD and intervention studies to reduce TMAO have been proposed to study this hypothesis [34].

There are several limitations of this study including small sample size and measurement of plasma TMAO only at a single time point on oral l-carnitine therapy. The findings in this study should be replicated in a larger study of patients with mitochondrial disease receiving oral l-carnitine.

In conclusion, the results of this study show that oral l-carnitine can markedly increase plasma TMAO in mitochondrial patients and therefore could increase risk of CVD over time. It is important to consider the potential harms of oral l-carnitine therapy especially given the limited evidence to date in support of health benefit in mitochondrial disorders. Further studies evaluating both the efficacy and long term safety of oral l-carnitine supplementation for the treatment of mitochondrial disorders are warranted.

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