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RESEARCH LETTER

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Carnitine consumption and effect of oral supplementation in human pulmonary arterial hypertension: A pilot study

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Abstract

Carnitine is required to transport fatty acid across the mitochondrial membrane to undergo beta oxidation. In addition to disorders of fatty acid metabolism, a relative carnitine deficiency has been reported in pulmonary arterial hypertension (PAH). Here we performed an observational study in which food and supplement consumption were collected in an observation period followed by open label administration of a carnitine supplement to determine feasibility of increasing plasma carnitine levels in humans PAH. We confirmed that relative carnitine deficiency in PAH is not due to reduced dietary consumption and that plasma levels of carnitine can be increased in PAH patients with supplementation that is well tolerated.

KEYWORDS
fatty acids, metabolism, pulmonary arterial hypertension

INTRODUCTION

Right ventricular (RV) failure is the most common cause of death in pulmonary arterial hypertension (PAH). A growing body of evidence suggests that metabolic abnormalities may underlie RV dysfunction in PAH.[1-5] Interventions against metabolic dysfunction in PAH may protect against or mitigate progression of RV failure.

We have identified abnormalities in fatty acid (FA) metabolism in PAH that overlap considerably with disorders of carnitine deficiency.[2,6] Carnitine, which is not synthesized by cardiomyocytes, links to an acyl group, which is required to transport FAs across the mitochondrial membrane to undergo beta-oxidation, the predominant source of ATP production in the human heart.[7] Inborn errors of carnitine metabolism and acquired carnitine deficiency are associated with cardiomyopathy.[6] Acquired deficiency primarily occurs via binding of carnitine to excess circulating fatty acids[6] as is found in PAH.[1] In human PAH plasma, we have shown a relative carnitine deficiency compared to healthy controls measured by increased acylcarnitine/free carnitine ratio.[8] We tested the effect of carnitine supplementation in a mouse

model of heritable PAH with RV failure that fully recapitulates the fatty acid metabolic defects found in human PAH. We found that carnitine improved RV contractility and ejection fraction by MRI, reduced RV lipid content and increased mitochondrial respiration, including long-chain fatty acid-stimulated respiration.[8] In humans with left heart failure, carnitine supplementation improves diastolic and systolic dysfunction and may reduce cardiovascular events.[9,10] While carnitine supplementation may have beneficial effects in cardiometabolic conditions, concern has been raised that carnitine metabolism by gut microbiota raises plasma concentration of pro-atherogenic trimethylamine-N-oxide (TMAO).[11] Whether carnitine supplementation can overcome relative carnitine deficiency in human PAH and potentially improve RV function is not known.

We performed an observational study in which food and supplement consumption were collected in an observation period followed by open label administration of a carnitine supplement to determine feasibility of increasing plasma carnitine levels in humans PAH. We hypothesized that carnitine consumption is normal in patients with PAH and circulating carnitine can be increased with oral supplementation. We further tested the effect of carnitine supplementation on TMAO plasma concentration in enrollees.

METHODS

Study design

We performed a prospective, single center study of dietary carnitine consumption and an open label study of carnitine supplementation (NCT04908397). This study was approved by the Vanderbilt University Medical Center IRB (#210899).

Participants

Ambulatory patients with Functional Class I-III idiopathic, heritable, associated with simple congenital heart disease or drugs/toxin use PAH, aged ≥18 years, stable on PAH therapy for ≥3 months before enrollment were eligible to participate. Patients with other PAH etiologies, eGFR <60 mL/min, or known allergy to L-carnitine supplements were excluded.

Study procedures

Study procedures are outlined in Figure 1a. Briefly, after informed consent, venipuncture was performed, and

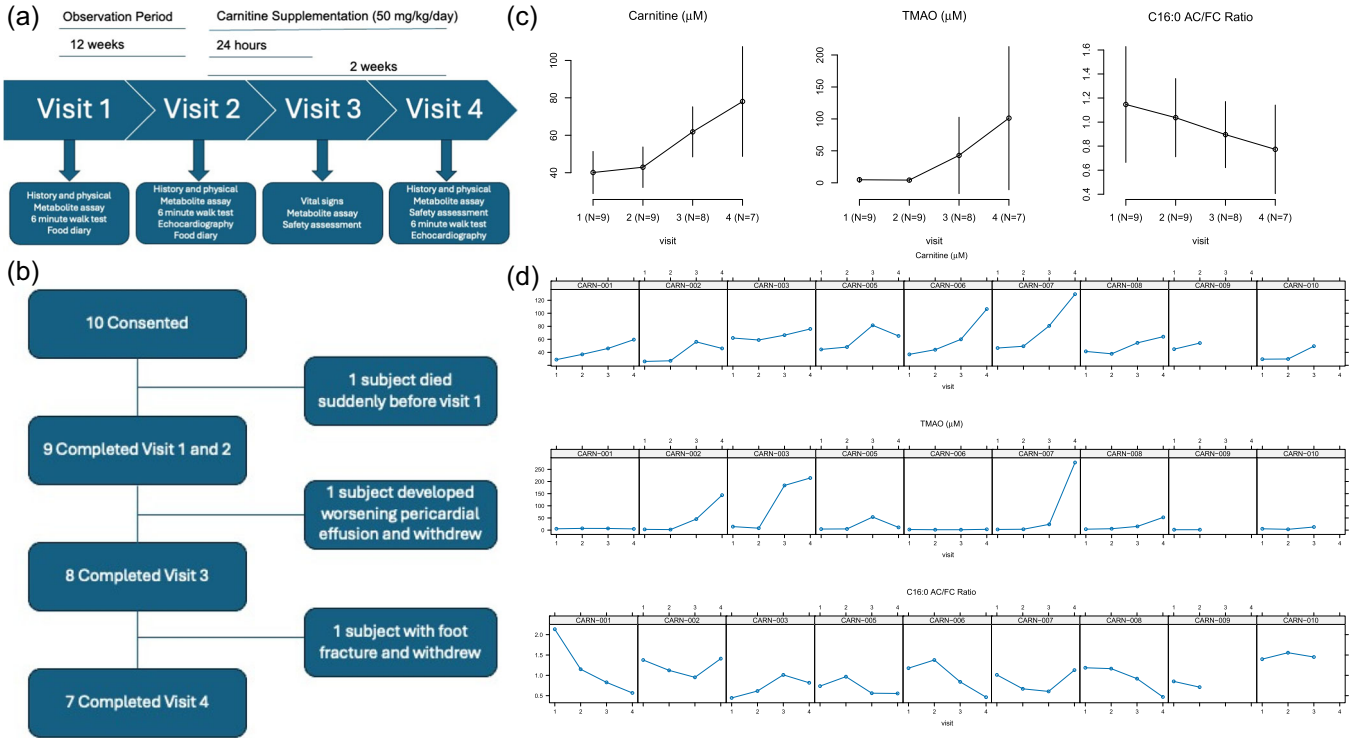

FIGURE 1 (a) Study design. (b) Participant disposition. (c) Aggregate plasma carnitine, trimethylamine-N-oxide (TMAO) concentration, and C16:0 acylcarnitine (AC): free carnitine (FC) ratio as measured by mass spectroscopy. (d) Individual plasma carnitine, TMAO and C16:0 ratio measured by mass spectroscopy.

participants recorded all food and nutritional supplements consumed for 3 days before Visit 1 using a validated tool (ASA24 Dietary Assessment Tool[12]) which also involved 6MWT, blood collection, and history and physical. Twelve weeks later, the food diary was repeated and participants underwent an echocardiogram, 6MWT, and blood collection (Visit 2). Carnitine supplementation (50 mg/kg/day[13]) was started after Visit 2 testing was complete. Participants returned twenty-four hours later for blood collection (Visit 3) and then after 2 weeks for repeat echocardiogram, 6MWT, and blood collection (Visit 4). Echocardiograms were analyzed by experienced readers (ELB and RM) blinded to timepoint. 6MWT was performed according to ATS guidelines.[14]

Metabolite assays

Carnitine, TMAO and acylcarnitines standards were purchased from Millipore Sigma (Burlington, MA). LC-MS/MS analysis was performed in a single batch after the last patient, last visit using a Thermo TSQ Quantum mass spectrometer interfaced to a Thermo HTC PAL refrigerated autosampler and a Thermo Surveyor HPLC pump.

Statistical analysis

Data reported as median (interquartile range) or mean (standard deviation) as appropriate. The primary endpoint of the supplementation protocol was change in plasma carnitine between Visits 2 and 4. Secondary endpoints included changes in acylcarnitine profiles, echocardiographic parameters, and 6MWT distance. Differences were analyzed using the Wilcoxon Signed Rank test and two sided $p < 0.05$ was considered significant. We made no adjustments for multiple comparisons in this exploratory study.

RESULTS

Enrollees

Nine participants completed the first study visit. Two participants did not complete the study protocol (Figure 1b) for reasons unrelated to the protocol or study drug. Enrolled participants were primarily idiopathic PAH, female (7/9 both) and aged 47.3 ± 9.3 years. Enrollees had long-standing disease with a mean time from diagnosis of 7.9 ± 5.7 years and all were functional class I or II with relatively preserved RV function (TAPSE 21 ± 4 mm). Five participants were using prostacyclin therapy (4/5 parenteral) and 8/9 subjects were on two or more therapies.

Dietary carnitine consumption

During the observational period, nine participants completed food diaries and plasma carnitine levels at Visits 1 and 2. All participants reported daily consumption of meat and/or seafood, four reported use of one or more non-carnitine supplements, and one reported use of a probiotic. There were no significant differences between baseline and 12-week plasma carnitine (baseline 41.5 [29.1, 45.8] and 12 week 44.0 [33.3, 51.8] μM, $p = 0.16$).

Drug tolerability and safety

Seven participants completed the full protocol with carnitine supplementation as planned for 2 weeks between Visits 2 and 4. One participant reported mild gastrointestinal symptoms.

Primary endpoint

Plasma carnitine concentration increased among protocol completers (44.0 [37.3, 48.7] μM–65.1 [61.8, 91.2] μM, $p = 0.016$, Figure 1c). Carnitine concentration increased numerically in all participants over 2 weeks but there was substantial variability in response (Figure 1d).

Secondary endpoints

Carnitine concentration was stable over the twelve-week observation period and increased after 1 day of administration from visit 2 to visit 3 in the eight participants completing these visits (40.9 [35.1, 48.4] μM vs. 58.1 [53.2, 70.0] μM, $p = 0.008$). The ratio of C16:0 acylcarnitine: free carnitine was numerically but not significantly reduced from visit 2 to visit 4 (1.14 [0.89, 1.22]–0.88 [0.77, 0.97], $p = 0.3$). TMAO plasma concentration, which was stable from Visit 1 to Visit 2, significantly increased from 4.80 [2.91, 6.42] μM–52.35 [8.11, 179.15] μM, $p < 0.05$) between Visits 2 and 4. There was marked elevation in three individuals while the remainder were minimally if at all changed (Figure 1d). There were no significant changes in echocardiographic measures of RV function including TAPSE, fractional area change, or RV global longitudinal strain. Similarly, there was no difference in NT-proBNP or 6 min walk distance between Visit 2 and 4.

DISCUSSION

We sought to understand dietary carnitine consumption in PAH including supplement use, to determine if plasma carnitine levels are stable over time and test the hypothesis that carnitine supplement can increase plasma carnitine levels in PAH patients. We further sought to examine if plasma levels of the pro-atherogenic metabolite TMAO are increased by carnitine supplementation. We found that enrollees ate a diet with adequate carnitine consumption and none used carnitine supplementation at baseline. Oral carnitine supplementation increased plasma carnitine at 24 h and 2 weeks while also increasing TMAO levels, particularly in three individuals. Carnitine supplementation was well tolerated and there were no adverse events.

Our study demonstrated the feasibility of carnitine supplementation in PAH and that relative carnitine deficiency may be rapidly overcome through this intervention. We were not powered to demonstrate improvement in clinically-important metrics such as 6 min walk distance, functional class, or echocardiographic markers of RV function. However, none of these metrics suggested that carnitine supplementation may be causing harm in PAH patients.

Prior epidemiologic data have suggested that TMAO, which is produced when carnitine or dietary nutrients such as choline are metabolized by gut microbiota to trimethylamine and converted in the liver to TMAO,[15] is proatherogenic and plasma levels predict incident cardiovascular disease. Emerging data support the hypothesis that TMAO plasma concentration may be associated with specific gut flora[15] that may be suppressed by pharmacotherapy.[16] Moutsoglou and colleagues have shown enrichment of gut microbial species with species that promote production of TAM and further demonstrated that TMAO plasma concentration was higher than healthy controls when stratified on the basis of REVEAL risk score.[17] Thus our data that carnitine supplementation in PAH increased otherwise stable levels of TMAO would be expected. While our sample size was too low to draw firm conclusions, the marked increase in TMAO plasma concentration in the single enrollee reporting use of a probiotic (CARN-003) does suggest that specific gut microbiota species may impact the production of TMAO in the context of carnitine supplementation. Future studies of carnitine supplementation in PAH may consider exclusion of individuals using probiotic supplements and inclusion of vegan or vegetarian diets to understand the implications of supplementation in different diets. Alternatively, given the relatively poor prognosis of PAH patients even in the modern era, the long-term effects of elevated TMAO may be less concerning. Our study included low-risk subjects with stable disease, which may have diminished the ability to detect differences in RV function after carnitine supplementation.

In conclusion, we confirmed that relative carnitine deficiency in PAH is not due to reduced dietary consumption, that plasma levels of carnitine can be increased in PAH with supplementation that is well tolerated. Further study is warranted to determine if carnitine supplementation may improve RV function in PAH while monitoring for adverse events mediated through TMAO.

AUTHOR CONTRIBUTIONS

Evan L. Brittain and Anna R. Hemnes contributed to study design, data acquisition, data analysis, drafting manuscript and critical editing. Alisha Lindsey, Kelly Burke, Ivan Robbins, Meredith Pugh, M. Wade Calcutt, Ravi Mallugari, James West contributed to data acquisition and analysis and provided critical editing. Hui Nian provided biostatistical support and contributed to data analysis and critical editing.

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CONFLICT OF INTEREST STATEMENT

The authors report no relevant conflicts of interest.

ETHICS STATEMENT

This study was approved by the Vanderbilt University Medical Center IRB (#210899).

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