The Uremic Metabolite, Trimethylamine-N-Oxide (TMAO), Directly Alters Cardiac Contractile Function

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INTRODUCTION

- TMAO is produced by intestinal microbial metabolism of phosphatidylcholine.
- Clearance of this uremic metabolite is dependent on urinary excretion.
- As kidney function declines during chronic kidney disease (CKD), plasma levels of TMAO increase. Studies in patients with chronic kidney disease (CKD) show the median serum TMAO concentration in end-stage renal disease patients is roughly 30-fold higher than individuals with normal kidney function.[1]
- Elevated plasma levels of TMAO show dose-dependent associations with the presence of cardiovascular disease including peripheral artery disease, coronary artery disease, myocardial infarction, and heart failure.[2,3]
- Cardiovascular disease accounts for more than 50% of the deaths in patients with end-stage renal disease.[4]

HYPOTHESIS

There have been no experiments to determine the *direct* effect of TMAO on cardiac muscle. Our general hypothesis is that TMAO has a direct effect on the cardiovascular system to promote cardiovascular disease. Our working hypothesis is that TMAO directly alters cardiac contractility via changes in cardiomyocyte intracellular calcium levels.

METHODS

- Adult CD1 male mouse hearts were dissected and the atria removed.
- Hearts were then suspended in oxygenated organ baths and paced at 1 Hz with bipolar stimulating electrodes.
- Muscle contractions to TMAO were measured using Powerlab hardware and LabChart data acquisition software (ADInsruments).
- To further explore a mechanism of action, rat embryonic cardiac myocytes (E18) were isolated and cultured for five days until confluent. Epifluorescence calcium imaging experiments were conducted using the calcium-indicator dye, Fluo-4.

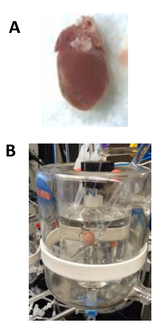

Figure 1. Panel A. Extracted CD1 male mouse heart**. Panel B.** Organ bath contractility apparatus. Hearts oxygenated and paced for experimentation.

RESULTS

TMAO Increases Isometric Force Production in Ventricular Cardiac Muscle

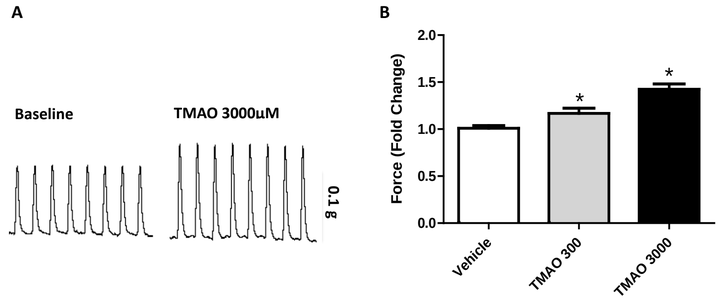

Figure 2. Panel A. Tracings of cardiac muscle contractions at baseline and following treatment with TMAO. **Panel B.** Acute treatment with TMAO increased average contraction amplitude of ventricular muscle 17% and 42% at 300 µM and 3000 µM, respectively. *denotes statistical significance from vehicle (n=6-7; P<0.05) using One way ANOVA with Bonferroni post hoc analysis

TMAO Increases Spontaneous Beating Frequency in Cardiac Myocytes

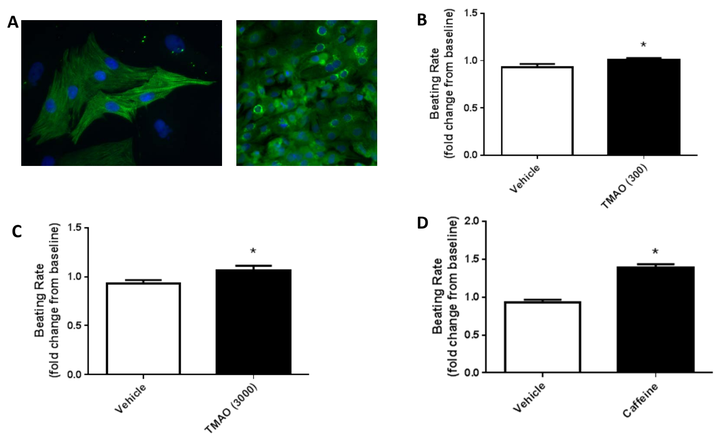

Figure 3. Panel A. In order to ensure cardiac phenotype, isolated rat embryonic myocytes were stained with two cardiac-specific markers, cardiac troponin I antibody (left image) and atrial natriuretic peptide antibody (right image). The cells were also stained with DAPI to identify the nuclei. Cardiac myocyte beating frequency was calculated before and after TMAO treatment at 300µM (**Panel B**), 3000 µM (**Panel C**), or 5mM caffeine (**Panel D**). This indicates that TMAO increased beating frequency similar to the calcium-releasing agent caffeine. * denotes statistical significance from vehicle (n=4-7, P<0.05) using a Student's T-T

SUMMARY

- TMAO, a uremic metabolite, is elevated during CKD but the direct effects on the cardiovascular system and specifically the heart are currently unknown.
- We have found that TMAO increases isometric cardiac contractile force.
- TMAO also increases the beating frequency of isolated cardiac myocytes, indicating TMAO likely increases intracellular calcium.

SIGNIFICANCE

- Our results are the first to demonstrate that TMAO directly increases cardiac contractility and frequency of spontaneous beating. While acutely these actions may be beneficial, chronic increases in intracellular calcium and cardiac stress promote cardiac remodeling and heart disease.
- Cardiovascular disease is the major cause of mortality associated with chronic kidney disease, yet standard interventions to modify the traditional cardiovascular risk factors (e.g. dyslipidemia, hypertension, hyperhomocysteinemia) have not significantly improved outcomes in this patient population.[5] Therefore, it is imperative that new therapeutic strategies are identified.
- TMAO may be a novel contributor in the development of heart disease in CKD and thus an important therapeutic target for reducing morbidity and mortality.

CREDIT & REFERENCES

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