The Metaorganismal Trimethylamine-Flavin Containing Monooxygenase 3-Trimethylamine N-Oxide Axis Regulates Endothelial Denudation After Arterial Injury And Promotes Vascular Smooth Muscle Cell Phenotype Switching

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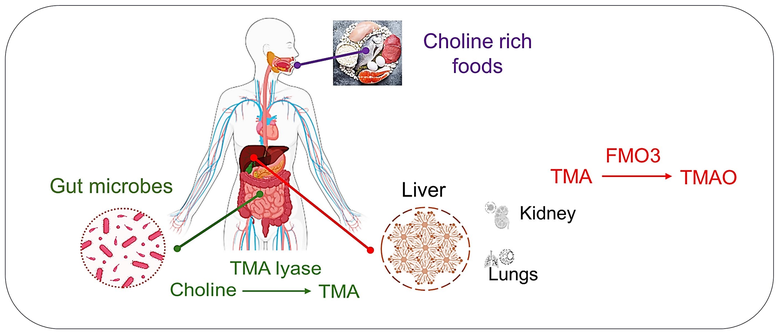
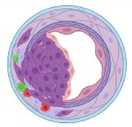
Background

The trimethylamine (TMA)-flavin containing monooxygenase-3 (FMO3)-trimethylamine N-oxide (TMAO) axis is dependent on diet, gut microbes, and host metabolism.





Neointimal hyperplasia after arterial injury

High choline diet and high TMAO is associated with greater restenosis after arterial injury

Cellular mechanism is not well understood

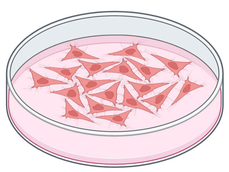
Hypothesis

TMA/TMAO will promote the migration of vascular smooth muscle cells (VSMCs), VSMC phenotype switching from a contractile to synthetic state, and increase neointimal hyperplasia after arterial injury.

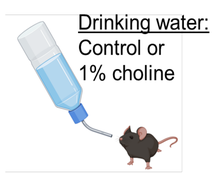
Methods

Human aortic smooth muscle cells (HAoSMC) *in vitro*

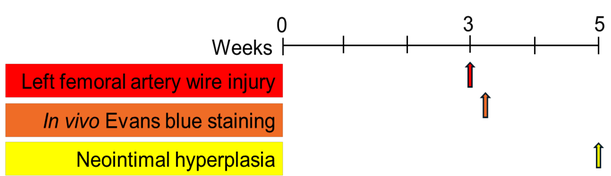
Treatment with 200 μM TMAO / 100 μM TMA / 10 ng/ml TGFB1



Female human liver-specific FMO3 overexpressing (FMO3-Tg) transgenic mice



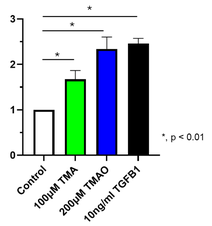
Drinking water:
Control or
1% choline



Results

TMA and TMAO increases VSMC cell viability in vitro

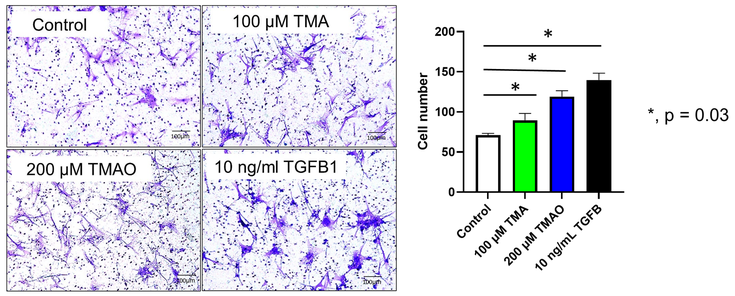
TMA increased 0.7-fold and TMAO increased 1.5-fold cell viability compared to controls at 48 hours in serum-free conditions.



Quantification of MTT assay at 48 hours. *n* = 4 per group, *p < 0.01.

TMA and TMAO increase VSMC migration in vitro

Cell migration was increased by 19% in TMA and 48% in TMAO treatments compared to controls in 6 hours.

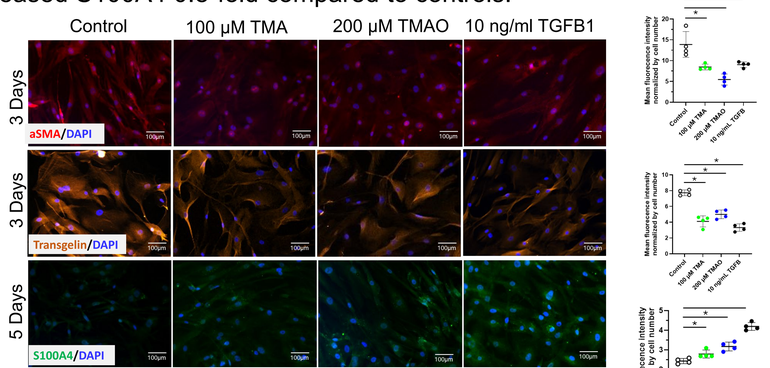


Left panel: Representative images of Transwell assay for cell migration. Right panel: Quantification of number of cells migrated in 6 hours, *n* = 3 per group, *p < 0.03.

TMA and TMAO decrease VSMC contractile marker and increase synthetic marker expression

Contractile markers: TMA decreased αSMA expression 5-fold and transgelin 4-fold. TMAO decreased αSMA expression 8-fold and transgelin 3-fold.

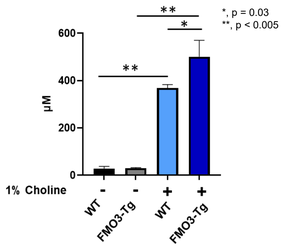
Synthetic markers: TMA increased S100A4 0.4-fold and TMAO increased S100A4 0.8-fold compared to controls.



Left : Representative images of immunofluorescence assay in VSMCs. Right : Quantification of mean fluorescence intensity normalized by cell number, *n* = 4 per group, *p < 0.03.

Dietary choline supplementation increases TMAO levels in FMO3-overexpressing transgenic mice

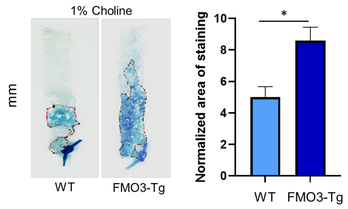
Choline treatment increased plasma TMAO levels in FMO3-Tg mice compared to wild-type (WT) controls.



Quantification of plasma TMAO levels in mice 2 weeks after injury, n=4 mice/group, *p < 0.03, **p < 0.005

Dietary choline supplementation delays endothelial recovery in FMO3-overexpressing transgenic mice

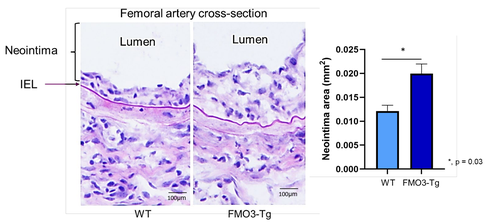
Evans blue staining area was greater in choline-fed FMO3-Tg mice (0.008 mm²) than WT controls.



Left panel: Representative Evans blue staining from injured arteries from wildtype (WT) and FMO3-Tg mice on Day 5 after injury. Right panel: Quantification of Evans blue staining area, *n*= 4 mice/group, *p < 0.03

Dietary choline supplementation increases neointimal hyperplasia in FMO3-overexpressing transgenic mice

Left: Representative arterial sections from choline-fed WT and FMO3-Tg mice 2 weeks after injury. Right : Morphometric analysis of neointima area showing 1-fold increase in neointimal area in FMO3-Tg mice compared to WT. *n*= 4 mice/group, *p = 0.03



Conclusions

- TMA and TMAO increase viability and migration of VSMC *in vitro*.
- TMA and TMAO decreased contractile and increased synthetic marker expression and induced a morphological change from spindle-shaped to hypertrophic in HAoSMCs *in vitro.*
- FMO3-Tg mice on dietary choline supplementation had greater plasma TMAO levels compared to WT, which was associated with delayed endothelial recovery and more neointima formation after surgical injury.
- Future studies will further understand the role of the TMA-FMO3-TMAO axis on VSMC and the arterial response to injury.