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Reduction of trimethylamine *N*-oxide to trimethylamine by the human gut microbiota: supporting evidence for '*metabolic retroversion*'

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Feeding trimethylamine *N*-oxide (TMAO; found in fish) to individuals with fish odour syndrome (FOS), in which trimethylamine (TMA) is not detoxified to TMAO by hepatic flavin-containing monooxygenases (FMOs), leads to conversion of 40–60 % of the oral dose of TMAO to TMA [1]. In these individuals, it is thought the gut microbiota reduces TMAO to TMA, and in those individuals without FOS the TMA is re-oxidized in the liver before being excreted in the urine. The process has been termed '*metabolic retroversion*' [1] (Fig. 1). We examined the bioconversion of TMAO to TMA by the microbiota using *in vitro* systems, and mouse models to determine whether '*metabolic retroversion*' of TMAO occurred *in vivo*.

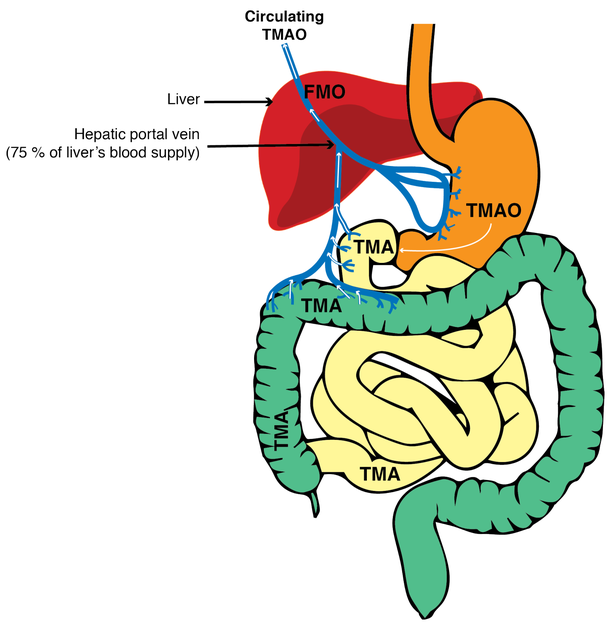


Fig. 1. '*Metabolic retroversion*': the reduction of TMAO to TMA by the gut microbiota, followed by oxidative reactions mediated by hepatic FMOs to regenerate TMAO [1]. TMAO is reduced by gut bacteria to TMA in the small intestine and proximal colon. TMA is taken up by the hepatic portal vein to the liver, where it is *N*-oxidized into TMAO by hepatic flavin mono-oxygenases (FMO) and demethylated into dimethylamine and monomethylamine by cytochrome P450s during first-pass metabolism. TMAO (and minor amounts of TMA) are excreted in the urine.

Strains (*n* = 66) of human faecal and caecal bacteria were screened on solid and liquid media [2] anaerobically at 37 °C for their ability to reduce TMAO, with metabolites in spent media analysed by ^{1}H-NMR. Effects of TMAO on mixed microbial growth was assessed using *in vitro* anaerobic, stirred, pH-controlled batch-culture fermentation systems inoculated with human faeces. Groups of 6-week-old C57Bl6/J mice were given free access to either tap water or tap water treated with a broad-spectrum antibiotic cocktail for 14 days. Mice were given in the morning by gavage either a solution of d_9-TMAO (Cambridge Isotope Laboratories, Inc.) or saline and euthanized 6 h later by cervical dislocation. Plasma samples were collected and stored at -80 °C. Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MS) was used to determine concentrations of TMA, d_9-TMA, TMAO and d_9-TMAO in samples [3].

TMAO significantly increased the growth rate of *Enterobacteriaceae* (Fig. 2a–c). While having no effect on the growth rate of lactic acid bacteria (Fig. 2d,e), TMAO had a significant effect on biomass produced by these bacteria (not shown) and led to increased production of lactate (Fig. 3). As only negligible levels of TMA were produced from TMAO by these bacteria, they were not using TMAO as an alternative electron acceptor in anaerobic respiration.

In mixed microbial populations, only the growth of *Enterobacteriaceae* was promoted in the presence of TMAO (Fig. 4a), with TMA produced only in the TMAO-containing systems (Fig. 4b).

In vivo '*metabolic retroversion*' of TMAO was confirmed in mice (Fig. 5), demonstrating that choline, phosphatidylcholine and carnitine [4–6] are not the only dietary sources of TMA with the potential to influence host metabolism.

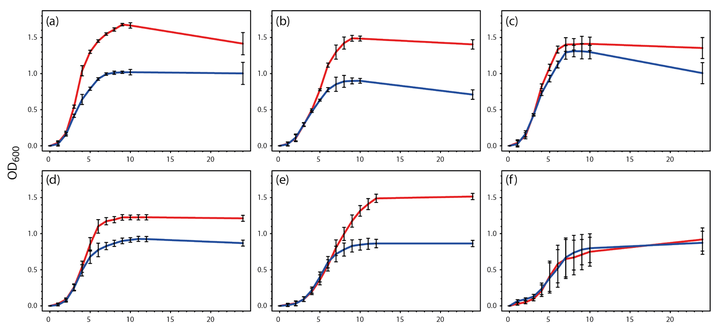
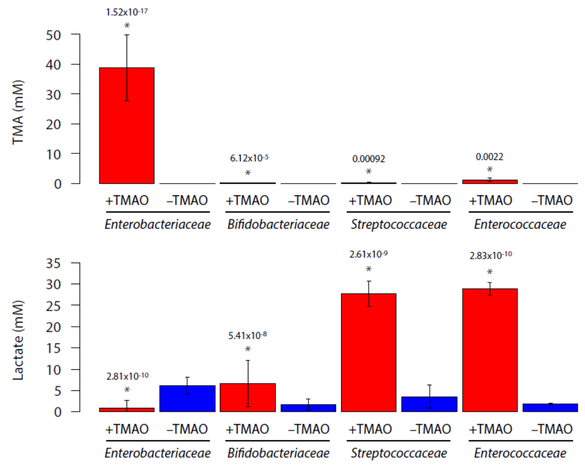
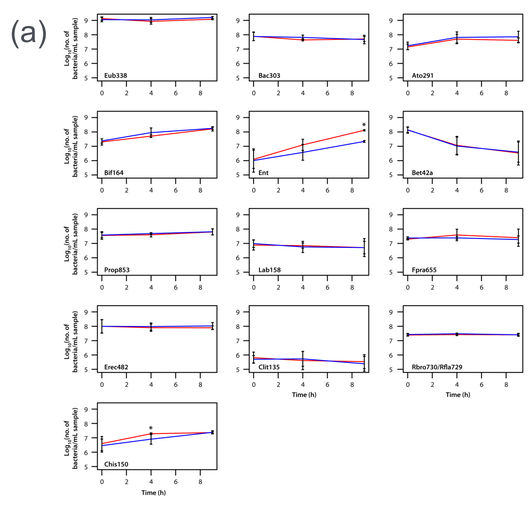


Fig. 2. Effect of TMAO on growth of gut bacteria. (a) *Escherichia coli*; (b) *Citrobacter gillenii*; (c) *Klebsiella pneumoniae*; (d) *Enterococcus gallinarum*; (e) *Streptococcus anginosus*; (f) *Clostridium perfringens*. Representative results are given (mean +/- SD, *n* = 3). Red, growth medium with 1 % TMAO; blue, growth medium without TMAO.

Fig. 3. Lactate production by lactic acid bacteria compared with that of members of the family *Enterobacteriaceae* in the presence of TMAO. *Enterobacteriaceae*, *n* = 20; *Bifidobacteriaceae*, *n* = 17; *Streptococcaceae*, *n* = 7; *Enterococcaceae*, *n* = 5. Two strains belonging to the *Lactobacillaceae* also produced increased amounts (6.96 ± 1.35 mM and 0.17 ± 0.07 mM, respectively) of lactic acid and TMA in the presence of TMAO (data not shown). Red, TMAO-containing medium; blue, negative control. *, Statistically significantly different (*t* test) from its negative control (adjusted *P* value < 0.05). Results are shown as mean +/- SD.





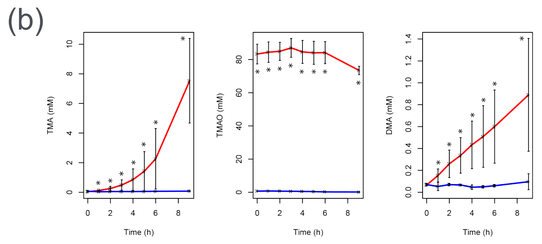


Fig. 4. Effect of TMAO on mixed faecal microbial populations *in vitro*. (a) Enumeration of bacteria in fermentation vessels by FISH analysis. (b) NMR data for batch culture samples. (a, b) Red lines, TMAO-containing systems; blue lines, negative controls. Data are shown as mean +/- SD (*n* = 3). *, Statistically significantly different (*P* < 0.05) from the negative control at the same time point.

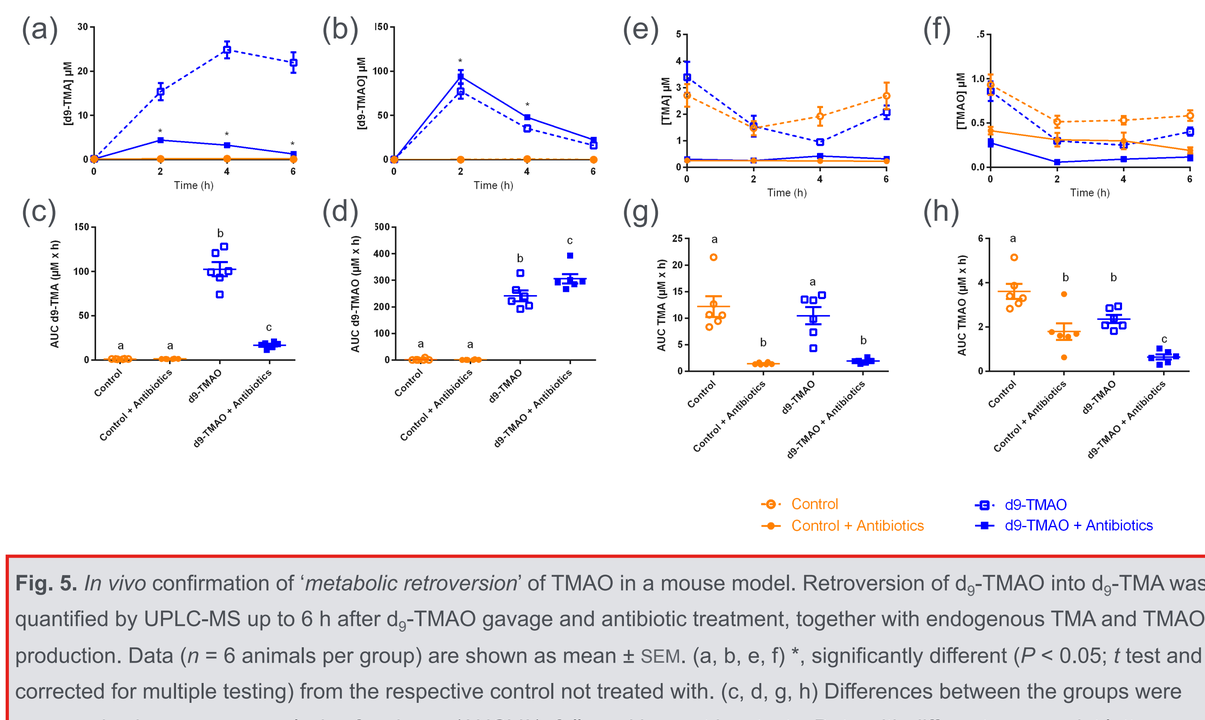


Fig. 5. *In vivo* confirmation of '*metabolic retroversion*' of TMAO in a mouse model. Retroversion of d_9-TMAO into d_9-TMA was quantified by UPLC-MS up to 6 h after d_9-TMAO gavage and antibiotic treatment, together with endogenous TMA and TMAO production. Data (*n* = 6 animals per group) are shown as mean ± SEM. (a, b, e, f) *, significantly different (*P* < 0.05; *t* test and corrected for multiple testing) from the respective control not treated with. (c, d, g, h) Differences between the groups were assessed using one-way analysis of variance (ANOVA), followed by post hoc tests. Data with different superscript letters are significantly different (*P* < 0.05). (a) Plasma quantification of post-gavage d_9-TMA. (b) Plasma quantification of post-gavage d_9-TMAO. (c) d_9-TMA bioavailability [area under the curve (AUC)]. (d) d_9-TMAO bioavailability (AUC). (e) Plasma quantification of post-gavage endogenous TMA. (f) Plasma quantification of post-gavage endogenous TMAO. (g) Endogenous TMA bioavailability (AUC). (h) Endogenous TMAO bioavailability (AUC).

In the absence of the microbiota, TMAO is taken up by the host into the circulation by an unknown mechanism. Elucidating this mechanism, and examining the effects of TMAO on lactic acid bacteria in greater detail are the focus of ongoing work.

*These authors made equal contributions to the work.

ACKNOWLEDGEMENTS

M.L.J.-P. was sponsored by Fundación Alfonso Martin Escudero (Spain). J.C. is funded by EU-FP7 METACARDIS (HEALTH-F4-2012-305312), M.-E.D. is funded by Nestlé, Institut Mérieux and EU-FP7 (METACARDIS). L.H. is in receipt of an MRC Intermediate Research Fellowship in Data Science (grant number MR/L01632X/1, UK Med-Bio).

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