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Commentary and Views

**Archaebiotics**

Proposed therapeutic use of archaea to prevent trimethylaminuria and cardiovascular disease

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Trimethylamine (TMA) is produced by gut bacteria from dietary ingredients. In individuals with a hereditary defect in flavin-containing monooxygenase 3, bacterial TMA production is believed to contribute to the symptoms of trimethylaminuria (TMAU; fish-odor syndrome). Intestinal microbiota TMA metabolism may also modulate atherosclerosis risk by affecting trimethylamine oxide (TMAO) production levels. We propose that reducing TMA formation in the gut by converting it to an inert molecule could be used to prevent or limit these human diseases, while avoiding the major drawbacks of other clinical interventions. Reducing TMA levels by microbiological interventions could also be helpful in some vaginoses. Particular members of a recently discovered group of methanogens, that are variably present in the human gut, are unusual in being apparently restricted to utilizing only methyl compounds including TMA as substrates. We confirmed experimentally that one of these strains tested, *Methanomassiliicoccus luminyensis B10*, is able to deplete TMA, by reducing it with H₂ for methanogenesis. We therefore suggest that members of this archaeal lineage could be used as treatments for metabolic disorders.

The Gut Origin of TMA in Human

The tertiary amine trimethylamine (TMA) is a volatile compound which has a characteristic fishy odor, and is formed by bacterial reduction of trimethylamine oxide (TMAO). This alkylamine is detected as unpleasant by the human olfactory system at even very low levels, thereby preventing humans from ingesting rotting fish. Ironically, this molecule is also endogenous in humans, being synthesized in the gut and sometimes in the vagina by the endogenous microbiota. The microbiome of the gastrointestinal tract is better characterized than that of other body sites, so only this niche will be discussed here, although relevant metabolic processes in the vagina are probably similar.

In the gut, TMA is formed by microbial conversion of dietary ingredients and is further absorbed before being oxidized into TMAO in the liver (see Fig. 1). Such TMA precursors include TMAO (in abundance in seafood), and choline (eggs, soybean, cauliflower), which is likely the most important TMA source. Recent data also show that L-carnitine found in red meat (and in some energy drinks and dietary supplements) is converted into TMA. The metabolic pathways leading to TMA are thought to be exclusively microbial in humans: the mechanism of conversion of choline by an anaerobic mammalian gut *Desulfovibrio* has recently been identified and relies on the cut (choline utilization) operon, in which cutC encodes a glycol radical enzyme with a choline trimethylamine-lyase activity. Choline is an important factor for human health, with an adequate intake for adults of 425 mg and 550 mg per day being recommended for women and men respectively in the US. Choline depletion

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**Keywords:** trimethylaminuria (TMAU), cardiovascular disease (CVD), TMA, TMAO, thermoplasma-related methanogens, archaeobiotics, choline, L-carnitine, vaginosis, *Methanomassiliicoccus* spp.

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resulting from a choline-deficient diet causes clinical consequences such as non-alcoholic fatty-liver disease (NAFLD) and muscle damage. Dietary lecithin (phosphatidylcholine, PC) also feeds into plasma TMAO levels by gut conversion into choline (and then to TMA), as shown by mouse studies. Thus, limiting intake of choline or its precursors to reduce TMA production is not feasible. However such a reduction of TMA levels is highly desirable because TMA is related to at least two disorders.

**TMA is Implicated in Disease**

Trimethylaminuria (TMAU) is caused by a metabolic anomaly whereby sufferers emit a pervasive fishy odor caused by the excretion of TMA in their breath, sweat and urine. As TMAU is still under-recognized and often goes undiagnosed, those affected often suffer from psychological problems and social stress. Although no large prevalence studies are currently available, people with compromised ability to N-oxidize trimethylamine into the odorless TMAO are probably common. TMAU can be classified into a primary genetic form and an acquired (or secondary) form. The genetic form is now well characterized with approximately 15% of single nucleotide polymorphisms (SNPs) in the FMO3 gene (encoding a flavin-containing monoxygenase) associated with either lack of or diminished enzymatic oxidation in the liver of TMA into TMAO. In the healthy individual, more than 95% of TMA is oxidized into TMAO in this way, while genetic defects in FMO3 result in accumulation of TMA in blood, and then in breath, sweat and urine (Fig. 1, pathway A). Acquired TMAU, sometimes transient, includes conditions featuring elevated urinary levels of TMA due to dietary and hormonal factors in combination with enzymatic activity and metabolism in the gut. Important examples include transient menstrual TMAU, overload of dietary precursors of TMA (see above), and impaired hepatic function. Treatment relies mainly on an empirical dietary approach, or several complementary therapeutic adjuncts, including soaps which limit the volatility of TMA from the skin, together with brief courses of antimicrobials (neomycin, metronidazole) which are not always effective. This last approach limits the bacterial load, and in extenso, gut conversion of TMAO, choline and derivatives into TMA.

Gut microbiota metabolism of phosphatidylcholine (PC) and L-carnitine can promote atherosclerosis. This is because their plasma metabolites (choline, betaine and TMAO) are risk factors for cardiovascular disease (CVD) in humans. Experimentally, dietary choline supplementation has been shown in a seminal paper to significantly enhance aortic lesion area in mice genetically prone to atherosclerosis, as did supplementation of the diet with TMAO. Furthermore, gut microbiota depletion by antibiotics led to inhibition of the dietary choline-induced cardiovascular effects. Mice with depleted gut microbiota did not experience increased TMAO plasma levels (Fig. 1, pathway B) nor did humans. Human gut microbiota is also required to form TMAO from L-carnitine, and plasma TMAO is likely the primary driver of cardiovascular risks rather than L-carnitine itself.

A fishy odor frequently occurs in bacterial vaginosis. It is important to stress that, in this instance, TMA does not originate from urine, but rather from in situ production of TMA by the vaginal microbiota through a process similar to that which occurs in the gut.

**Hypothesis: That TMA Could Be Depleted as it is Synthesized**

We were prompted by recent microbiology studies to ask if TMA could be depleted in vivo by bioconversion into a molecule with no undesirable properties. The underlying biochemistry was described more than 35 years ago in the rumen of cows, where choline is metabolized via TMA and onwards into methane. Rumen microbes like the methanogenic archaea Methanosarcina barkeri metabolize methyl compounds, including TMA, to methane for growth. A distinct archaeal group (the Rumen Cluster C; RCC) was subsequently identified as being putatively able to convert methyl compounds including TMA and methyamines into methane. Half the human subjects examined to date also produce methane, usually by harboring the methanogen Methanobrevibacter smithii, or less frequently, Methanospirillum hungatei (reviewed in ref. 15). It is also noteworthy that M. smithii has been identified in vaginal samples. We thus asked if methylothrophic methanogens would be able to survive and deplete TMA in the human gut (or in the vagina), a notion that would be greatly supported if methanogens that naturally occur in the human gut could metabolize TMA.

The trigger for proposing this hypothesis now is that, until recently, only the methanogens *M. smithii* and *M. stadtmaneae* were recognized as residents of the human gut. They belong to the order Methanobacteriales, one of the six known orders of methanogens. However in 2008, the existence of a seventh order inhabiting the human gut (hereafter referred to as the Mx-lineage) was proposed, particularly common in older subjects, and *Methanomassiliicoccus luminyensis*, the first and unique isolated member of this order metabolizes methanol with hydrogen for methane production. Genomic sequences of three of these unusual methanogens are currently available, that of *M. luminyensis*, and genome sequences that we recently determined for *Candidatus Methanomethylophilus alvus* and *Candidatus Methanomassiliicoccus intestinalis* grown in an enrichment culture from human stool. "*Ca. Methanomethylophilus alvus*" belongs to the RCC cluster that was recently highlighted for its putative involvement in TMA consumption in the rumen, while *M. luminyensis* and "*Ca. Methanomassiliicoccus intestinalis*" are phylogenetically closely related to the RCC cluster. These three genomes have all of the genes necessary for reduction of methanol using hydrogen, but also for the reduction of tri-dimethylamine with hydrogen (Fig. 2), while the two other possible pathways for methanogenesis (CO~2~ reduction with H~2~ and acetoclastic methanogenesis) are genetically incomplete. Whereas the capacity of these strains to reduce methanol with hydrogen was previously demonstrated, their ability to grow on TMA with hydrogen has not been investigated. We therefore tested this hypothesis on the sole strain of this group.
available in pure culture, *M. luminyensis* B10, and thus confirmed for the first time that this strain is able to grow on TMA with hydrogen (Fig. 3). *M. luminyensis* B10 was also able to grow on the by-products of TMA catabolism, dimethylamine and then monomethylamine, both being used with hydrogen for methanogenesis (data not shown). These results are in agreement with the annotated genetic complement of this strain and strongly suggest the same metabolic capacity in "Ca. *M. alvus*" and "Ca. *M. intestinalis*" because they harbor orthologs of all the relevant *M. luminyensis* genes involved in these pathways. Collectively this suggests to us that natural methanogenic inhabitants of the human gut will be able to metabolize TMA, and could deplete this metabolite as it is formed by bacterial elements of the microbiota.

**Proposal: The Archaebiotics Concept**

Drawing together these ecological and biochemical strands, we now propose the Archaebiotics Concept viz. the intestinal application of specific archaeal strains for the treatment of human diseases including cardiovascular disease and TMAU (Fig. 1). The fishy-odor aspect of some vaginal conditions could be addressed using pessary delivery. Focusing on CVD and TMAU, there are several advantages. Methane, the TMA-metabolite produced by these archaea is usually considered biologically inert in humans. The pathway and relevant coding sequences for catalyzing TMA conversion to methane by "Ca. Methanomethylophilus alvus" and "Ca. Methanomasiliicoccus intestinalis" based on annotation of the genomes we recently sequenced is shown in Figure 2; a similar pathway is inferred from the *M. luminyensis* genome. As for
other methanogenic archaea, it includes a methyltransferase MttB with a predicted pyrrolysine residue,
which is associated with its synthetic machinery. The fact that these strains were isolated from humans
might facilitate regulatory compliance. M. luminensis like M. smithii is susceptible to bacitracin, metronidazole, ordiazole and squalamine,
providing the reassurance of contingency eradication. An alternative to administering live cells might be therapeutic
administration of the relevant enzymes (Fig. 2), all of which are also encoded by M. luminensis.

Paralleling the experience with the development of probiotic bacterial strains for human consumption, it has been
shown that particular archaenal methanogen strains are likely to be more suitable for clinical use in this application. For
example, we established that some strains seem to be evolutionarily more adapted to the human gut ecosystem, harboring
genes predicted to confer resistance to bile salts (bile salt hydrolases occur in “Ca. Methanomethylophilus alvus” species, but not in M. luminensis nor in “Ca. Methanomasillus intestinalis”). Consideration of factors other than TMA depletion alone is thus appropriate (see also below).

**Challenges for Therapeutic Usage for Archaea to Deplete TMA**

Technological hurdles to overcome will include establishing the best way to deliver oxygen-sensitive microorganisms into the human gut, and whether or not this administration will lead to cell levels that are sufficient to allow clinically relevant TMA depletion. Continuous administrations may be necessary if subjects do not become colonized. There are additional biological challenges; will these strains be able to effectively deplete TMA in the gut? Are the ensuing risk reductions for cardiovascular disease achieved, as they were by antibiotic therapy to limit TMA production? It is also important to concede that archaenal abundance is not uniformly associated with increased health in mammals; for example, Bacteroides thetaiaotomicron- M. smithii co-colonization of germ-free mice increased host adiposity.

Archaea in general appear to
be more abundant in native Africans than in African Americans, likely indicating dietary influences on the microbiota. Diet could conceivably be controlled or modulated to promote methanogen levels in recipients of archaebiotics. We cannot currently explain why the Mx-lineage of Archaea appears from the literature to be more abundant in older subjects, so we are determining fecal archaeal levels in the ELDERMET cohort subjects, that are well phenotyped for diet, health and bacterial microbiota. A possible factor is the trophic interaction with other microbiota elements, and that a more favorable ecology emerges in some older subjects. This raises the provocative issue of what the effects of introducing a methanogenic archaea might be on the microbiota of the recipient. The Mx-methanogens are very likely not acetoclastic but would compete with other hydrogenotrophic archaea and bacteria, so the downstream effects on microbiome, metabolome and host physiology would need detailed exploration in vitro, in animal models, and ultimately in humans. Although the application of archaebiotics as proposed here faces significant challenges, we contend that the anticipated benefit of their use to mitigate the life-time risk of CVD and to treat TMAU warrants a concerted research effort in this area by the scientific community.

Figure 3. Growth of Methanomassiliicoccus luminyensis strain B10 on trimethylamine (TMA) and hydrogen (H2). (A) The growth of M. luminyensis B10 on methanol (M, 50 mM) and H2 (previously described by Dridi et al.) was used as a positive control for growth and maximal cell density. (B) We now show that M. luminyensis B10 also grows on TMA (15 mM) in the presence of H2 (filled diamonds) but not on TMA in the absence of H2 (open diamonds). Accordingly, TMA is depleted in presence of H2 (filled triangles) and not in the absence of H2 (open triangles). The composition of the gas phase was measured at the end of the experiment (C) and revealed the production of methane (CH4) and the depletion of H2 in presence of either M or TMA. No CH4 was produced in the absence of H2 and CH4 was not produced nor H2 depleted in the absence of the methylated compound substrate (M or TMA). M. luminyensis B10 was obtained from DSMZ (DSM No. 25720) and cultivated in DSMZ medium 119, with rumen fluid replacing the sludge fluid. When H2 was present, initial atmosphere composition was: N2/H2/CO2 (55:35:10), and in absence of H2: N2/CO2 (75:25). Growth in each condition was performed in triplicate. Trimethylamine concentration was measured as described by Krätzer et al. OD, Optical Density.
Disclosure of Potential Conflict of Interests
No potential conflict of interest was disclosed.

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References
1. Zhang AQ, Mitchell SC, Smith RL. Dietary precur-
3. Craciun S, Balskus EP. Microbial conversion of
4. Institute of Medicine (U.S.). Standing Committee
6. Spencer MD, Hamp TJ, Reid RW, Reid RW, Fischer LM, Pump PM. Trimethylaminuria: causes and diagno-
7. O'Toole PW, Harris HM, Peyret P, Brugère JF. Methylotrophic methan-
9. O'Toole PW, Harris HM, Tottey W, Mihajlovski A, Fardeau ML, Ollivier B, Raoult D. Complete genome sequence of
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