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A pomegranate extract inhibits the conversion of dietary L-carnitine to prothrombotic trimethylamine (TMA) by the gut microbiota

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Trimethylamine N-oxide (TMAO) is a cardiometabolic risk factor(1) and can cause prothrombotic effects in humans(2). L-carnitine is a major dietary precursor of TMAO, which is metabolised by the human gut microbiota to γ-butyrobetaine (γ-BB) and finally to trimethylamine (TMA). In the liver, TMA is converted to TMAO. Several interventions have been suggested to reduce the microbial production of TMA, including broad-spectrum antibiotics(3) and the choline analogue 3,3-dimethyl-1-butanol (DMB)(4). However, the effectiveness of broad-spectrum antibiotics wears off after 6 months, and they may contribute to antimicrobial resistance [3]. For DMB, its suppressing effects could not be replicated in later studies(5).

The aim of this study was to investigate the potential of a polyphenol-rich pomegranate extract to inhibit TMA production from the dietary precursor L-carnitine by the gut microbiota.

In vitro fermentation models were inoculated with 1% faecal inoculum from different healthy donors (n = 4, ClinicalTrials.gov registration number NCT02653001), together with 2 mM L-carnitine, and different doses of a pomegranate extract (5.7, 11.4, or 22.8 mg/mL). The batch fermentations were carried out under colonic conditions (anaerobic using a continuous N^2 flow, pH 6.66–7.12, and 37°C). Additionally, a 96-well plate- format fermentation model(6) was carried out in an anaerobic cabinet at 37°C to screen the effect of various polyphenols present in the extract, including ellagic acid and punicalagin. Samples were collected from both models and at various timepoints over 48 hours. The concentrations of L-carnitine, γ-BB, TMA, and related metabolites were quantified in the samples using LC-MS/MS. Differences in concentrations of metabolites were confirmed by means of Two-Way ANOVA.

The pomegranate extract showed a dose-dependent inhibitory effect on the metabolism of L-carnitine to γ-BB and TMA, with significantly higher L-carnitine concentrations at 10 and 12 h after inoculation for all doses ($p < 0.001$) and at 20 h for the highest dose ($p < 0.01$). TMA appeared in all models between 12–20 h but tended to be at lower concentrations in the pomegranate-treated models ($p = 0.06$, highest dose). Moreover, there was a high inter-individual variation in the production of TMA between donors. Upon initial screening of the isolated polyphenols, higher concentrations of L-carnitine were found at 8 h when treated with punicalagin compared with the control ($p < 0.05$).

These findings demonstrate that the pomegranate extract suppressed L-carnitine conversion to γ-BB and TMA. This may subsequently reduce the levels of the prothrombotic TMAO. Prospectively, further screening of the (combined) TMA-suppressing effect of isolated polyphenols will be carried out. Additionally, a pharmacokinetic human study will be performed to investigate the pomegranate extract as a suppressive agent for the microbial production of TMA from L-carnitine, while metagenomic studies will be undertaken to identify the structural changes of th microbiome evoked by the extract.

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