

### Impact of Protein Sources in a Guideline-Based Omnivorous Diet on Circulating Biogenic Amine Metabolites: A Randomized Controlled-Feeding Trial

Samitinjaya Dhakal,<sup>1</sup> Moul Dey,<sup>1</sup> and Zahra Moazzami<sup>2</sup>

<sup>1</sup>South Dakota State University and <sup>2</sup>University of Minnesota

**Objectives:** Age is a risk factor for chronic diseases—the leading causes of death and disability. An omnivorous diet compared to a plant-based diet was shown to increase blood Trimethylamine-N-oxide (TMAO)—a biogenic amine biomarker for atherosclerosis that may also increase the risk for insulin resistance and certain cancers. Worldwide, pork and chicken are the most popular animal proteins, however, poultry is perceived as healthier. It is unknown if pork intake differentially modifies the TMAO-response than poultry which was investigated here using a non-inferiority study design to avoid any potential negative results.

**Methods:** In a randomized, active-controlled, 2-arm-crossover trial (NCT03539666), healthy adults ( $n = 38/\text{arm}$ , age  $\geq 50$  y) consumed 156 g/day of lean pork or white-meat chicken as the main protein source as part of an omnivorous diet based on the 2015 USDA dietary guidelines. The feeding arms were matched for food ingredients, nutrients, and energy. Overnight-fasted samples were

collected before and after each diet phase. LC/MS with isotope-labeled internal standards and 16S-rDNA sequencing were used for metabolite and microbiome measurements, respectively. Univariate and multivariate data were analyzed using supervised and unsupervised machine learning algorithms in R.

**Results:** A non-inferiority of pork to poultry for a circulating TMAO-response (97.5% CI, linear mixed model) was observed for the first time. In addition, global biogenic amine signature (346 metabolites, 95% CI), microbiota-dependent essential TMAO precursor—trimethylamine (97.5% CI), three dietary TMAO substrates (choline, betaine, and L-carnitine) as well as the microbiota composition (95% CI) underlying the observed TMAO-response were not different between the two proteins. TMAO phenotype varied at baseline; a higher baseline circulating TMAO concentration associated with distinct microbiota profiles (all, FDR corrected  $p < 0.05$ ) and greater TMAO-response ( $p = 0.0001$ ) independent of the dietary proteins.

**Conclusions:** Lean pork does not exacerbate TMAO-risk in omnivores 50 years or older. With 95% of Americans being omnivorous and a third of them fast aging, lean pork increases dietary protein options that may help improve adherence to dietary recommendations for greater healthspan.

**Funding Sources:** National Pork Board, National Institute of Food & Agriculture