

Heme-induced colorectal carcinogenesis associated with meat consumption: relationship between fecal microbiome, metabolome and luminal heme-induced lipoperoxidation activity in rats

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Background: Recently, the WHO classified processed meat and red meat consumption respectively as «carcinogenic» and «probably carcinogenic» to humans. Heme iron of meat plays a pivotal role in the promotion of both preneoplastic and tumoral lesions in mice by enhancing luminal lipoperoxidation. Alteration of gut microbiota by antibiotic treatment prevented heme-induced lipoperoxidation, suggesting a role of the gut bacteria in the carcinogenicity of heme iron.

Aim: We aimed to identify fecal bacterial communities and metabolites modulated by heme intake and/or related to end-products of lipoperoxidation.

Methods: Groups of 8 F344 rats were fed for 22 days with 4 experimental AIN76 diets supplemented or not with, hemin (1.5 $\mu\text{mol/g}$), calcium carbonate (2%) or hemin+calcium carbonate. Diets were balanced for protein, fat and iron (0.036%). Heme concentration was assessed by fluorescence and lipid peroxides were quantified by TBARs measurement in fecal water. Genotoxicity on colon mucosa was evaluated by alkaline comet assay. 18 gut bacterial communities were monitored by qPCR using GUT Low Density Array approach and metabolomic profiles of the same fecal homogenates were obtained through ^1H NMR spectroscopy. Integration of data sets was performed using the R package mixOmics.

Results: As expected, increase of fecal TBARs resulting from hemin-enriched diet (81.1 \pm 17.9 vs. control 37 \pm 10.4 μM , $p < 0.0001$) was prevented by the supplementation of calcium in hemin-enriched diet (44.2 \pm 10.6 vs. control, ns), luminal heme being chelated by calcium carbonate. DNA mucosal damages were associated with elevated fecal TBARs levels, suggesting that chelation of heme by addition of calcium was sufficient to normalize both heme-induced lipoperoxidation and associated mucosal genotoxicity. Regularized canonical correlation analysis highlighted similar structuration of data sets from fecal microbiome and metabolome, consisting in segregation of relative abundances of bacterial groups and metabolites according to diet (**Figure 1**). *Enterobacteriaceae* and *Bacteroides fragilis* were enriched in microbiota of rats fed with hemin whereas *Roseburia spp.* and *Lactobacillus spp.* were underrepresented. Addition of calcium in hemin-enriched diet normalized heme-induced increase of *B. fragilis* and reduction of *Lactobacillus spp.*, suggesting that these bacterial groups were correlated with heme-induced lipoperoxidation. In parallel, fecal short chain fatty acids (acetate, propionate and butyrate), as well as succinate and several bile acids, covaried with lipoperoxidation status.

Conclusion: This dietary study in rat provides a global overview of heme-induced modulations within fecal bacterial communities and metabolites and identifies those that are closely linked with lipoperoxidation and genotoxicity status, two hallmarks of meat consumption and colorectal cancer risk.

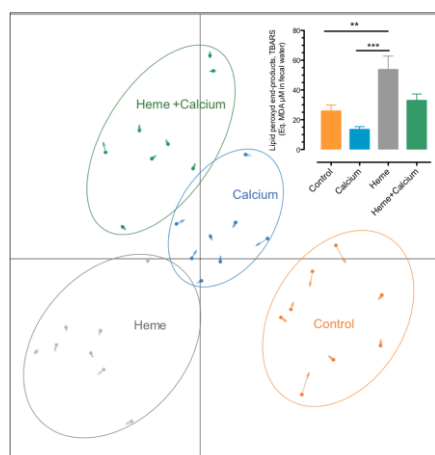


Figure 1: Arrow plot illustrating integration of fecal microbiome and metabolome data sets according to diet using rCCA analysis. Each arrow represents a rat for which arrow start from the coordinates in the microbiome-subspace to the coordinates in the metabolome-subspace (n=8). Lipid peroxid end-products in fecal water. TBARs are expressed as equivalent of MDA (μM in fecal water).