UNTARGETED METABOLOMICS OF COLONIC DIGESTS REVEALS KYNURENINE PATHWAY METABOLITES, DITYROSINE AND 3-DEHYDROXYCARNITINE AS RED VERSUS WHITE MEAT DISCRIMINATING METABOLITES

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i) Introduction and Objectives

Epidemiological research has demonstrated that the consumption of **red meat** is associated with **colorectal cancer (CRC)**, **diabetes mellitus** and **cardiovascular diseases**. The main hypothesis states that heme iron, present in red meat, can catalyze the formation of **toxic N-nitroso compounds** (NOCs) and **lipid peroxidation products** (LPOs) (Bouvard *et al*., 2015). However, **further research is required** to fully elucidate the exact working mechanisms. Moreover, involvement of the **gut microbiome** in red meat-associated diseases has been proposed (Allison-Silva *et al*., 2016).

ii) Materials and Methods

To gain holistic insight in metabolites that are formed during the colonic digestion of red meat, an **untargeted mass spectrometry (MS) based metabolomics approach** (Vanden Bussche *et al*., 2015) was used on **colonic meat digests**. The **human gastro-intestinal digestion was simulated** with **beef and chicken meat** (control) and for the colonic fermentation, microbiota were obtained from fecal inocula of ten healthy volunteers and incubated separately. Data preprocessing and multivariate statistical analysis was performed to retain only important red meat-associated metabolites. For identification, online databases were consulted (HMDB and MetFrag) and analytical standards were purchased. Additional ***in vitro*** **digestions** were performed with **carnitine** and **heme-containing myoglobin** to mechanistically support the formation of the identified metabolites.

iii) Results and Discussion

Data preprocessing resulted in 4686 ions (3908 and 778 in + and – ionization modes, respectively). After multivariate statistical analysis, 287 red meat-associated metabolites were retained, of which for 78 an identity could be annotated (HMDB). Finally, based on structural fragmentation and/or purchased standards twenty-two red meat-associated colonic metabolites were identified. For five of these an involvement in red meat-associated diseases could be hypothesised. Additionally, the required red meat components for their synthesis were confirmed. The **formation** of **N-formylkynurenine, kynurenine, L-kynurenic acid** (kynurenine pathway metabolites) and **dityrosine** was **heme-dependent** and **carnitine** was demonstrated as the **precursor of 3-dehydroxycarnitine.**
Kynurenine pathway (KP) metabolites have been linked to the development of **cancer** and **diabetes mellitus** (Noto *et al.*, 1978; Goldstein *et al*., 2000; Heng *et al*., 2016). **Dityrosine** is a marker for **oxidative stress**, a heme-catalyzed process that is also involved in the development of cancer (Oliver *et al*., 1975). Trimethylamine-N-oxide (**TMAO**), hepatically formed out of 3-dehydroxycarnitine, has been linked to **atherosclerosis** (Koeth *et al.,* 2014). Evenmore, it is possible that **TMAO** is also involved in the **onset of CRC**, since elevated levels of this metabolite in plasma are associated with higher incidence of CRC in women (Bae *et al.,* 2014).
The used innovative MS-based metabolomics platform proved to be a powerful platform in the understanding of the causal relationship between red meat consumption and associated diseases.

Theme: ‘The gut and beyond’