MICROBIOTA AND HOST

Microbiota and Host (2024) **2** e240006
https://doi.org/10.1530/MAH-24-0006

Received 22 July 2024
Accepted 3 October 2024
Available online 3 October 2024
Version of Record published 6 November 2024

RESEARCH

The gut microbiome without any plant food? A case study on the gut microbiome of a healthy carnivore

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Abstract

Objective: The carnivore diet is a ketogenic diet based exclusively on the consumption of food of animal origin. While the impact of various diets on the gut microbiome is extensively documented, the effects of a carnivore diet remain unclear. To address this gap, we conducted a pilot study on the gut microbiome of an individual following a carnivore diet and compared it with that of a subgroup of healthy individuals.

Methods: A stool sample was collected from a healthy 32-year-old male adhering to a carnivore diet and was sequenced using 16S DNA Amplicon Sequencing. The results were then compared to those from three control groups possessing similar anthropometric characteristics and differing in their frequency of meat consumption.

Results: The gut microbiome of the carnivore was dominated by the phylum Firmicutes and the genera *Faecalibacterium, Blautia*, unspecific *Lachnospiraceae*, *Bacteroides*, and *Roseburia*—bacteria known for fiber degradation. Furthermore, neither alpha- nor beta-diversity, nor the functional capacity of the gut microbiome, showed differences when compared to the control groups. Additionally, the gut microbiome of the carnivore showed the least similarities with the microbiome of the cohort consuming meat on a daily basis.

Conclusion: In our study, we showcase the compositional and functional characteristics of the gut microbiome in an individual on a carnivorous diet, finding no differences in comparison to a control cohort. Further research is needed to investigate the short- and long-term impacts of a carnivorous diet on gut health through cross-sectional and longitudinal studies.

Significance statement:

To the best of our knowledge, this is the first study to report on the composition of the gut microbiome of a person adhering long-term to the carnivore diet.

Keywords: carnivore; carnivore diet; fiber; gut microbiome; plant-based diet; prebiotics

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Introduction

The carnivore diet is characterized by the exclusive consumption of animal products, including meat, offal, eggs, butter, and dairy products such as milk, cheese, kefir, and yogurt. Recently, it has gained popularity as an extension of the ketogenic and Atkins diets, with many adherents reporting benefits such as increased energy, improved skin condition, and overall better health, as evidenced by a 2021 survey analysis (Lennerz *et al.* 2021). However, while the beneficial effects of the ketogenic and modified Atkins diets on conditions such as epilepsy and obesity are well documented (Foster *et al.* 2003, Johnston *et al.* 2014, Martin-McGill *et al.* 2020, Sondhi *et al.* 2020, Devi *et al.* 2023, Manral *et al.* 2023), the health impacts of the carnivore diet remain uncertain.

Analyzing the gut microbiome is a valuable method to portray the profound influence of specific diets on human health. The gut microbiome, a complex and dynamic community of microorganisms including bacteria, archaea, fungi, and viruses, performs essential functions that contribute to the host's health (Human Microbiome Project Consortium 2012*a*,*b*). Diet is a significant factor affecting both the composition and function of the gut microbiome. The benefits of fiber-rich (Wu *et al.* 2011, Ghosh *et al.* 2020) and plant-based diets, such as the Mediterranean diet (Meslier *et al.* 2020, Zhu *et al.* 2020), are well-established, as are the detrimental effects of Western diets high in processed foods and fats (Shen *et al.*, 2014, Cho *et al.* 2017, Koeth *et al.* 2019, Wolf *et al.* 2020, Wu *et al.* 2020, Dhakal *et al.* 2022, Guthrie *et al.* 2022, Teigen *et al.* 2022, Whelan *et al.* 2024). Conversely, evidence suggests that short-term, fiber-reduced, and ketogenic diets may also benefit the gut microbiome (Basciani *et al.* 2020, Van den Houte *et al.* 2024). However, studies on Western-style diets, which are rich in animal products but also high in processed foods, are not directly comparable to the potential effects of the carnivore diet, which lacks processed foods and may differently impact gut health (Chassaing *et al.* 2022, Sellem *et al.* 2024). Therefore, it is crucial to investigate the gut microbiome within the context of the carnivore diet to understand its effects and to inform dietary recommendations.

To date, to our knowledge, only one study has examined the gut microbiome in a strictly animal-based diet, focusing only on its short-term effects (David *et al.* 2014). To address this gap, we conducted a case study comparing the gut microbiome of an individual on a carnivore diet for a total of 4 years with three other cohorts characterized by different frequencies of meat consumption.

Methods

Study design and setting

The study was conducted at the Gut Microbiome Center (CCM) in Zagreb, a privately owned institution specializing in gut microbiome research and analysis. The pilot study was intended to be conducted as an observational, cross-sectional study. The recruitment was performed during the year 2024 via social media and networks in the researcher's environment. Inclusion criteria were adult age, adherence to a carnivore diet for at least 12 months, and informed consent. Exclusion criteria were pregnancy; absence of inflammatory, infectious, or autoimmune diseases of the digestive tract; gluten intolerance; chronic medication; intake of probiotics or antibiotics less than 3 months before study inclusion; obesity; and illiteracy.

Health assessment

The intent was to objectify the diet and the health status by different methods. The plan was to initially perform an interview with the carnivore subject with a medical doctor and dietitian to assess health status by anamnesis and medical examination, medical history, lifestyle, and dietary habits, with a focus on food intake. Additionally, two self-reported questionnaires assessing dietary intake from two perspectives (athlete diet and Mediterranean diet) were employed. Secondly, the subjects were to undergo a laboratory analysis of venous blood (EDTA or citrate containers) and urine samples for conventional diagnostic parameters of immunity and metabolism (blood count, lipid panel, hormones, and vitamins). Glucose metabolism would be monitored over the course of 10 days with a wear-on device.

Gut microbiome analysis

The gut microbiome was analyzed by the 16S rDNA amplicon sequencing method. Stool samples were taken using a home test kit with a cotton swab of toilet paper following the instructions of BIOMES NGS GmbH. The samples were transported by logistical services the next workday over the course of a couple of days to the laboratory (Biomes NGS, Wildau, Germany). Upon arrival, the stool samples were stored at –20° C until sequencing. The sequencing method and bioinformatic analysis are following protocol, discussed in detail elsewhere (Pfeil *et al.* 2023, Siptroth *et al.* 2023*a*,*b*).

Comparison with omnivores

To obtain a better understanding of the carnivore's gut microbiome a comparison with omnivore controls was performed. The data bank of Biomes NGS Ltd. from Wildau, Germany, containing the data of the gut microbiome and health status and lifestyle, assessed by an online self-report questionnaire, of several thousand individuals living in Europe was sourced for adequate controls. All individuals gave written consent for the scientific use of the given data. The gut microbiome analysis of all fecal samples, including those of the carnivore was performed in the same laboratory, so bias resulting from the sequencing process and bioinformatic analysis could be eliminated.

The selection criteria for the controls were based on the traits and habits of the carnivore subject, such as age, gender, health status, and lifestyle habits. The aim was to restrict the criteria as much as possible, to ensure the exclusion of as many as possible confounding factors. These were the specific selection criteria for the controls:

- 27–37 years old
- male sex
- no diseases (self-reported)
- no gastrointestinal complaints self-reported
- no gluten intolerance self-reported
- no chronic medication self-reported
- no antibiotic intake in <3 months self-reported
- no probiotic intake <3 months self-reported
- BMI 18.5–35 kg/m^2
- alcohol intake not more than 3–4x per week
- non-smoking
- good self-reported wellbeing (≥5 out of 10)
- good self-reported health score (≥ 6 out of 10)
- physical activity (training) ≥ 3 times/week (including low, middle, and high intensity)

To improve the comparison between the carnivores and the omnivores, the retrieved individuals were stratified into three cohorts according to their self-reported meat consumption: one of daily meat eaters, one of regular meat consumers (≥ 3 times/week meat consumption), and one of rare and no meat consumers (<3 times/week meat consumption). Meat intake as a pillar of the carnivore diet was deemed a discriminant factor regarding the gut microbiome.

Since the data on relative taxa and pathway abundances were not normally distributed median, interquartile range (IQR), and minimal and maximum values were calculated. Since the median of certain taxa reached near-zero values, the respective taxa's abundance was equated to zero to facilitate further analysis.

Results

The study was conceived as a pilot study on the topic of gut microbiome composition and functionality in individuals with long-term adherence to a carnivorous diet. The recruitment took place between April 2023 and April 2024 via social media and networks in the researcher's environment. Inclusion and exclusion criteria are disclosed in the Methods section. After a yearlong search only one subject adhering to a carnivore diet willing to participate in such a study was found. Only one individual met the inclusion criteria. Therefore, we regard the following study as a case study, acknowledging the potential biases that may arise from this approach. The individual was willing to participate in the study which he confirmed by signing an informed consent form regarding all undertaken measurements.

The carnivore

A comprehensive anamnesis of the individual's current health status and medical history was obtained through interviews conducted by a medical doctor and a dietitian.

Diet

The individual is a 32-year-old man. On the day of sample collection, he had been on a carnivorous diet for 4 years, consuming exclusively meat, butter different types of cream, a variety of hard cheeses, and eggs. Although he consumes dairy in the form of cheeses and sour cream, he did not consume other fermented dairy products such as kefir, yogurt, or similar products. He declared that the motivation for the carnivore diet to be neither health nor mindset related. The subject reports enjoying preferably food encompassed by this specific dietary regime (meat, butter, eggs) and that it facilitates his everyday life (groceries, meal preparation).

His daily diet consists of mostly lean meat (beef, veal) and pork (daily), roasted or cooked, hard cheese and butter, and less poultry (2–3x times a week) as assessed in the interview with the dietitian. Occasionally, he tests food containing carbohydrates due to professional reasons, but this occurs less than once a month and does not exceed more than a spoon of food containing carbohydrates. He reports not having consumed any vegetables, fruits, grains, or legumes for the time period of the last 4 years. The subject supplies his meat from a local butcher, and it is mostly grown in non-industrial conditions, while he purchases dairy mostly in the form of butter and hard cheeses from small-scale dairy producers. A more detailed 7-day meal registry is included in Supplementary material 1-Survey results (see section on supplementary materials given at the end of this article).

General health

After initiating it experimentally more than 4 years ago, he has experienced several beneficiary effects (dermatological status, gastrointestinal functioning) which have been encouraging him to further pursue this dietary regime. The subject supposedly is not experiencing any adverse effects of this diet and has been functioning exceptionally in everyday life. The subjects proclaimed being perfectly content with their life and health status. His medical history is unremarkable, without any registered complaints and he has not been taking any medication, dietary supplementation, or any probiotics during the 3 months leading up to the date of stool sampling. He also reported having no nutritional intolerances or allergies.

The subject has a background in bodybuilding for several years and is currently having 3–4 training weight-lifting sessions lasting at least 60 min weekly. Using the 6-tiered Participant Classification Framework for the level of physical activity and sports performance, the subject was classified as a Tier 2 athlete (McKay *et al.* 2022). The subject has been a non-smoker for his whole life and refrains from alcohol consumption. He lives in a flat in a city with a companion and is self-employed.

Health assessment

The body composition of the test person was assessed by bioimpedance analysis (MC-780MA, Tanita, Tokyo, Japan), and the data are disclosed in Table 1.

To objectify the dietary intake two unrelated self-reported questionnaires were utilized in online form: The Athlete Dietary Index (ADI) (Capling *et al.* 2019) and the Mediterranean Diet Adherence Screener (MEDAS) (García-Conesa *et al.* 2020). The individual scored low on both questionnaires, 38% on the ADI and 2/14 on the MEDAS, which indicates that his diet differed substantially from actual guidelines for an athlete and Mediterranean diet (Supplementary Material 1-Survey Results).

Table 1 Laboratory analyses.

	Carnivore	Ref. interval
Blood count		
Erythrocytes, 10^9/L	5.23↑	3.86–5.08
Leukocytes, 10^{12}/L	4.7	3.4–9.7
Thrombocytes, 10^9/L	271	158–424
Hemoglobin, g/L	162↑	119–157
Neutrophils, %	55.1	44–72
Lymphocytes, %	33.4	20–46
Physiology		
Sodium, mmol/L	138	137–146
Potassium, mmol/L	4.1	3.7–5.4
Urea, mmol/L	6.8	2.8–8.3
Creatinine, µmol/L	105↑	49–90
Uric acid, µmol/L	392↑	134–337
Total cholesterol, mmol/L	4.5	<5.0
HDL, mmol/L	1.6	>1.2
LDL, mmol/L	2.6	<3.3
CK, IU/L	227↑	10–153
LDH, IU/L	165	25–241
Hormones		
Vitamin D, nmol/L	123.8↑	25.4–123.0
Vitamin B12, pmol/L	929↑	156–672
Folic acid, nmol/L	26.42	>12.19
Cortisol, pmol/L	710.8↑	145.4–619.4
TSH, mIU/L	1.96	0.55–4.78
Inflammation		
CRP, mg/L	0.5	0.2–5.0
Urine		
pH	5.5	4.5–8.0
Ketones	Positive	n.a.

CK, creatinine kinase; CRP, C-reactive protein; LDH, lactate dehydrogenase; TSH, thyroid-stimulating hormone.
↑indicates above reference interval

Laboratory analysis

An extensive laboratory examination was carried out to assess the test person's state of health. Laboratory analyses were performed at a tertiary health care facility where the subject was registered in an outpatient setting. Blood and urine samples were collected in the morning hours rested and prior to food intake at one time-point. A selection of results of the laboratory analyses relevant to this study are disclosed in Table 2. All results not disclosed in Table 2 were within reference intervals (Supplementary material 2-Laboratory analysis). A slight elevation of erythrocytes and hemoglobin levels was observed, which cannot be attributed to hemoconcentration due to normal electrolyte values. Increased creatinine, uric acid, and creatinine kinase levels are seen, with normal cholesterol levels. Creatinine clearance was estimated using the Cockroft-Gault formula and indicated normal renal function (Table 2). A significant increase in B12 levels and minimal increases in cortisol and vitamin D levels are observed as well. The urine was slightly acidic and positive for ketone bodies which could be attributed to ketoacidosis. This and the significantly high values of B12 could be seen as the only abnormal laboratory values.

Continuous glucose monitoring

For 14 days the participant wore a continuous glucose-monitoring device. The CGM device utilized is developed by Libre View, Abbott Ltd, Abbott Park, IL, USA. The organization's profile on the Libre View platform was utilized to monitor and evaluate the recordings, jointly with the reports on food intake measured with quantitative food frequency questionnaires sampled over the course of a week. The average glucose level was 3.5 mmol/L, the participant was 94% of total time in target range glucose values, and the glucose variability was 25%. One could conclude based on the continuous glucose monitoring that the patient had very well-regulated blood glucose levels.

Table 2 Shannon indices of omnivore cohorts.

	Cohorts		
	Daily	High meat	Low meat
Median	7.74	7.78	7.76
First quartile	7.37	7.40	7.45
Third quartile	8.11	8.11	8.10
Interquartile range	0.74	0.70	0.65
Minimum	7.36	7.42	7.44
Maximum	8.11	8.13	8.09

Gut microbiome comparison

To obtain a better understanding of the carnivore study subject's gut microbiome, a comparison with omnivore controls was performed. Healthy omnivores were selected out of the data bank of Biomes NGS Ltd. from Wildau, Germany, applying the inclusion and exclusion criteria listed in the Methods section. The criteria were based on the traits and habits of the study subject to minimize the role of potential confounding factors. By applying these criteria, a total of 151 individuals from the data bank were retrieved. Since the controls were stratified based on meat consumption, those 151 individuals were grouped into three cohorts (daily, high, and low meat consumption). The first cohort of daily meat eaters consisted of seven subjects, the second or high meat consumption cohort of 68 subjects, and the third or low meat consumption cohort of 76 subjects. Our hypothesis is that the gut microbiome of the carnivore, regarding taxonomic composition and functionality will bear the most similarities to the first two cohorts, and the least with the low or no meat consumption cohort. Meat consumption is a key dietary component of the carnivorous diet and is also considered a discriminating factor for the gut microbiome.

The Shannon–Weiner Index indicating α-diversity was 7.91, the inverse Simpson's Diversity Index, an alternative, 6.71. The gut microbiome composition on phyla and taxa levels is disclosed in Fig. 1.

The most abundant phylum is by far and hence dominant phylum is Firmicutes (75%), followed by important constituents such as phylum Bacteroidetes (11%) and phyla Proteobacteria and Actinobacteria (around 6.5% each). The dominance of Firmicutes is illustrated by a high Firmicutes-to-Bacteroidetes ratio of 6.64. On level of genera the gut microbiome is dominated by genera *Faecalibacterium*, *Blautia*, unspecific *Lachnospiraceae*, *Roseburia*, *Fusicatenibacter* from phylum Firmicutes, genera *Bacteroides* from phylum Bacteroidetes and genus *Bifidobacterium* from phylum Actinobacteria, which account for more than 50% of the total gut microbiome composition.

Comparison with omnivores

Since no sound conclusions on the nature of the carnivore's gut microbiome can be made solely on its own, the data were compared with omnivores selected by the process disclosed above.

Diversity

To evaluate the carnivore's gut microbiome diversity α- and β-diversity was assessed along the compared cohorts. For α-diversity the values of the Shannon Index of the carnivore and the controls were compared (Fig. 2). Detailed data on the value of the Shannon indices of the three cohorts is disclosed in Table 2. The carnivore's value is above the median of all three cohorts but within the interquartile range of all three cohorts. The α-diversity of the carnivore's gut microbiome does not behave as an outlier, on the contrary, it is regular, slightly above the expected value for an individual of his age, health status, and lifestyle. The carnivore's gut microbiome is as diverse as those of omnivore individuals.

To further strengthen this observation, β-diversity was calculated for all subjects comprised within the study. PCA values were based on Aitchison distance. Here again, the carnivore did not behave as an outlier but more as a regular sample from an individual of this age, health status, and lifestyle (Fig. 3). Although two samples (one from the high and one from the low meat consumption cohort) differed extremely regarding beta-diversity, the carnivore was very close to the 0-axis, indicating a great number of shared taxa with samples of all three cohorts, regardless of meat consumption.

Taxa

For the comparison of the taxonomic composition between the carnivores and omnivores, only the genus level was considered in the framework of this study. A descriptive statistical analysis of the genus abundances in the three cohorts was performed (Supplementary material 3 comparison results), and the resulting

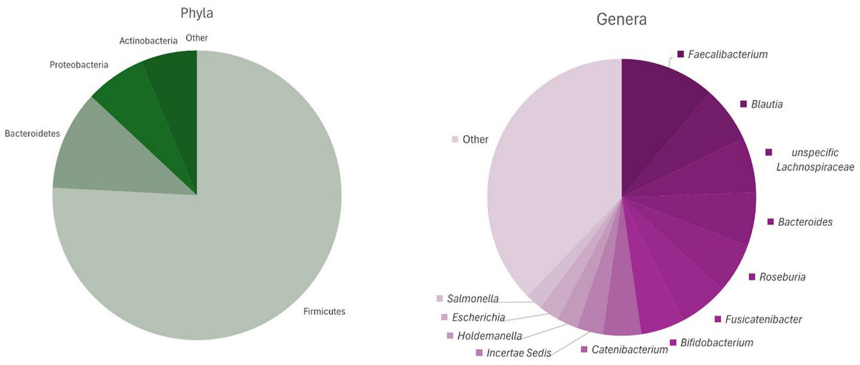

Figure 1

Carnivore's gut microbiome composition on phyla and genera level.

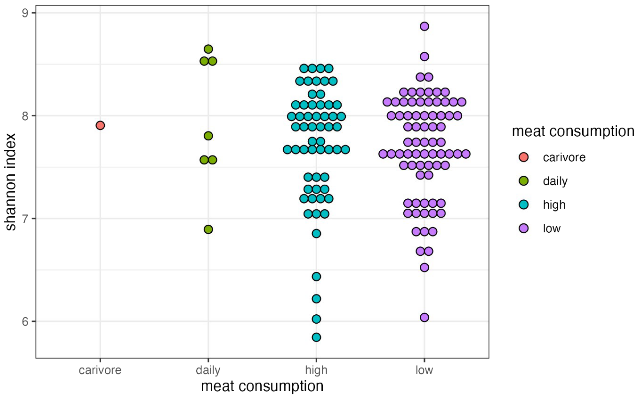

Figure 2

α-diversity comparison.

medians and IQR were used for the comparison with the carnivores. To exclude false-positive results, values of abundances below 0.5% were set to zero.

To simplify the comparison of the gut microbiome composition between the carnivores and omnivores, only the greatest differences in abundance were examined. Genera where the carnivore's abundances were below the 10th or above the 90th percentile in at least one of the three cohorts were included in a heatmap (Fig. 4). The heatmap colors of the omnivore cohorts represent the genus median within the respective cohort.

The most prominent differences (<10th; >90th percentile) regarding the abundance of genera were seen in 23 genera, disclosed in Table 3.

Considering the dominance of the phylum Firmicutes in the gut microbiome of the carnivore, it follows that the highest number of distinct genera identified (n = 17) are members of this phylum.

When compared to omnivores the carnivore's gut microbiome harbored greater abundances of 11 genera and lesser abundances of 6 genera belonging to Firmicutes. Of those, greater and lesser, seven genera were members of family *Lachnospiraceae* (*Blautia*, *Fusicatenibacter*, *Incertae Sedis*, unspecific *Lachnospiraceae*, *Lachnospiraceae NK4A136 group*, *Anaerostipes*, *Lachnospiraceae* UCG-004, CAG-56), indicating that this was by far the most distinct family of bacteria of the carnivore when compared to the omnivores.

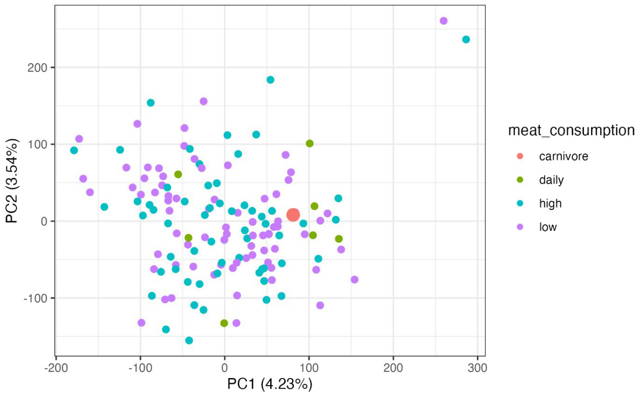

Figure 3

β-diversity of the carnivore and omnivore controls.

When looking at the most dominant genera among the carnivore's gut microbiome nine of them, belonging to the phyla Firmicutes, Actinobacteria, and Proteobacteria could be regarded as particular for the carnivore's gut microbiome. Their values differed the most from all three cohorts, regardless of meat consumption. In descending order according to relative abundance they are *Blautia*, unspec. *Lachnospiraceae*, *Fusicatenibacter*, *Incertae Sedis*, *Holdemanella*, *Catenibacterium*, *Bifidobacterium*, *Escherichia*, and *Salmonella.*

Other genera were also detected, but to provide a better overview, the focus was on genera with the highest abundance compared to the omnivore cohorts, as well as the dominant genera in the carnivore gut microbiome.

Functionality

Functionality was analyzed by comparing the abundance and activity of the predicted functional composition with PiCRUST2 (Douglas *et al.* 2020) between the control groups and the carnivores. A total of 190 metabolic pathways were predicted from the identified bacterial taxa. Similar to the analysis of taxa, we examined the results regarding the metabolic pathways in the carnivore's gut microbiome that were most aberrant compared to the median values of the respective metabolic pathways of the cohorts.

Due to the differences in gut microbiome functionality between the carnivore and the other cohorts, we used higher percentile ranges as benchmarks. For the comparison, we included pathways for the carnivore below the 1st percentile and above the 99th percentile of any of the omnivore cohorts (Fig. 5). Despite the more extreme cut-off values compared to the genus analysis, we identified an exceptionally high number of 126 distinct pathways. Since it is impractical to examine all these divergent pathways, only general observations are presented.

To better understand the functional dynamics of the different microbial communities, we grouped the predicted metabolic pathways predicted into functional modules using MetaCYC (Caspi *et al.* 2016, 2020) based on their class, ontology, or interpretation. The carnivore's functional profile according to the metabolic modules was compared with the median values of the omnivore values (Fig. 6, Table 4).

The function modules are listed according to the values of the carnivores in descending order. No major discrepancy was noted regarding the carnivore's

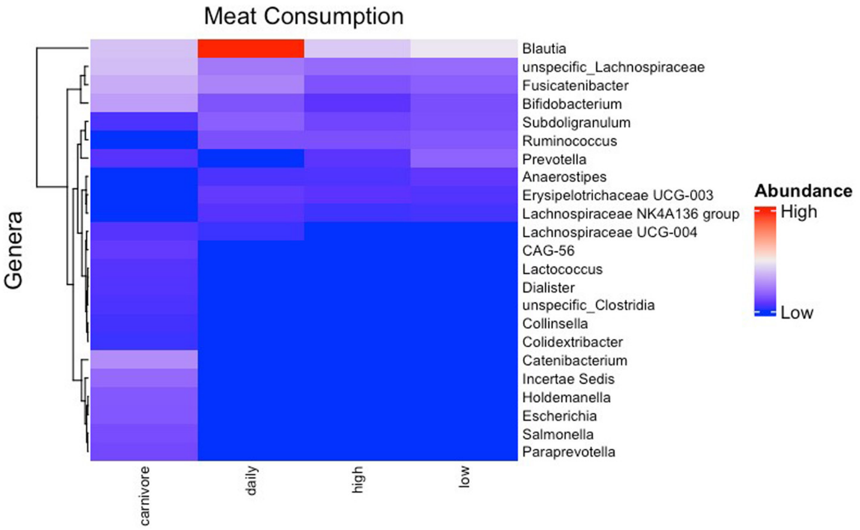

Figure 4

Most aberrant genera.

scores on the functional modules, especially regarding ‘protein fermentation’, ‘appetite and cholesterol level’, and ‘constipation factors’. The carnivore’s scores are bigger than the median scores of all three cohorts in the modules ‘energy metabolism and hyperacidity’ which includes pathways related to microbial lactate metabolism, ‘fructose intolerance’, ‘vitamin K production’, ‘inflammation indicators’, ‘gut barrier function’ and ‘cytotoxins’. Since the cohorts’ values on the modules between ‘vitamin K production’ and ‘cytotoxins’ were relatively low, the carnivore’s scores were proportionally 25% to over 300% bigger on those modules. On the other hand, was the carnivore’s score in the module ‘carbohydrate degradation (polysaccharides)’ slightly lower than those of the omnivore cohorts.

Table 3 Distinct genera of the carnivore gut microbiome.

Phylum	Genus	Cohorts			Carnivore
		Daily	High	Low	
Proteobacteria	*Escherichia*	0.009	0.009	0.02	2.443↑
Proteobacteria	*Salmonella*	0	0	1.023	2.072↑
Actinobacteria	*Collinsella*	0.136	0.220	0.208	0.626↑
Actinobacteria	*Bifidobacterium*	2.316	1.216	2.595	5.246↑
Bacteroidetes	*Prevotella*	0.027	1.196	2.91	1.112↑
Bacteroidetes	*Paraprevotella*	0.430	0.034	0.081	1.921↑
Firmicutes	unspecific *Clostridia*	0.328	0.003	0.273	0.812↑
Firmicutes	*Colidextribacter*	0.183	0.119	0.109	0.520↑
Firmicutes	*Dialister*	0.079	0.292	0.157	0.970↑
Firmicutes	*Lactococcus*	0.002	0.002	0.004	1.059↑
Firmicutes	*Incertae Sedis*	<0.001	0	0	3.451↑
Firmicutes	*Catenibacterium*	<0.001	0.003	0.002	4.570↑
Firmicutes	unspecific *Lachnospiraceae*	3.776	3.216	1.274	6.450↑
Firmicutes	*Holdemanella*	0.002	0.013	0.017	2.499↑
Firmicutes	*Fusicatenibacter*	4.214	2.248	2.767	5.820↑
Firmicutes	*Lachnospiraceae* UCG-004	0.520	0.386	0.399	1.091↑
Firmicutes	CAG-56	0.044	0.222	0.203	1.416↑
Firmicutes	*Subdoligranulum*	2.753	1.805	2.222	0.855↓
Firmicutes	*Ruminococcus*	2.183	1.237	2.481	0.293↓
Firmicutes	*Blautia*	16.757	6.903	8.054	6.618↓
Firmicutes	*Lachnospiraceae NK4A136* group	1.122	0.567	0.687	0.325↓
Firmicutes	*Anaerostipes*	0.817	0.889	1.364	0.323↓
Firmicutes	*Erysipelotrichaceae* UCG-003	1.375	1.193	0.974	0.058↓

↑values higher than the cohorts; ↓values lower than the cohorts

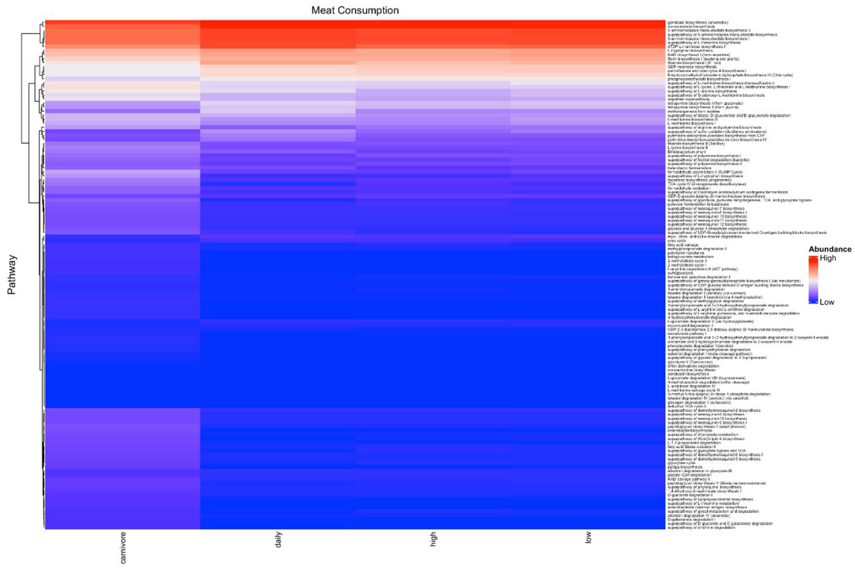

Figure 5

Most aberrant pathways.

Discussion

The present study was the first to our knowledge to assess gut microbiota composition and functionality of a subject adhering to the carnivore diet in scientific literature. The subject was young, male, regularly physically active and did not report any health-related complaints. His good health status was confirmed by an extensive laboratory analysis which, besides certain minor deflections from reference values, confirmed only ketoacidosis and elevated vitamin B12 levels, but normal cholesterol levels and no vitamin deficiencies. Through continuous glucose monitoring and assessment of metabolic laboratory parameters, no abnormalities in the subject's glucose metabolism were found. The carnivore was indeed healthy in general terms.

The carnivore's gut microbiome was on the phylum level dominated by Firmicutes, with substantial proportions of phyla Bacteroidetes, Proteobacteria, and Actinobacteria. On the genus level, 12 genera constituted the majority of the gut microbiome: 8 genera from phylum Firmicutes (*Faecalibacterium*, *Blautia*, *Roseburia*, *etc.*), genus *Bacteroides* from phylum Bacteroidetes, genus *Bifidobacterium* from phylum Actinobacteria, and genera *Escherichia* and *Salmonella* from phylum Proteobacteria.

To gain a better understanding of the carnivore's gut microbiome, it was compared with the data on the gut microbiome of 151 healthy omnivores, selected according to criteria to eliminate confounding factors, and stratified into three cohorts based on meat consumption: daily, high, and low. The healthy controls did not report any medical complaints, chronic illnesses, or chronic use of medications in an online questionnaire when providing their stool sample. The carnivore's gut microbiome was expected to be most similar to those of the daily and high meat consumption cohorts.

But the comparison showed surprising results. The carnivore's gut microbiome did not stand out regarding α- and β-diversity, indicating that it did not lack richness or diversity when compared to its omnivore counterparts. Regarding its taxonomic and functional profile, distinctive differences were detected, but they were not smaller between the higher meat consumption cohorts and the carnivore than the low meat consumption cohort. The differences in all three cohorts regarding metabolic pathways were far more drastic than those regarding taxa.

When compared with omnivores, the most abundant distinctive genera of the carnivore's gut microbiome were *Blautia*, unspec. *Lachnospiraceae*, *Fusicatenibacter*, *Incertae Sedis*, *Holdemanella*, *Catenibacterium*, *Bifidobacterium*, *Escherichia*, and *Salmonella.* When looking at the functional modules, the intestinal microbiome of carnivores was disproportionately

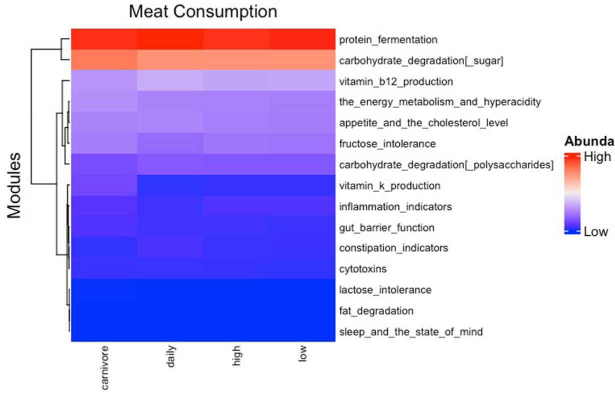

Figure 6

Comparison of functional modules.

Table 4 Comparison of functional modules.

	Cohorts			
	Daily	**High**	**Low**	**Carnivore**
Protein fermentation	0.081	0.077	0.079	0.078
Carbohydrate degradation (sugar)	0.061	0.061	0.061	0.066
Vitamin B12 production	0.028	0.027	0.027	0.024
The energy metabolism and hyperacidity	0.020	0.020	0.019	0.022
Appetite and the cholesterol level	0.020	0.019	0.019	0.020
Fructose intolerance	0.016	0.018	0.017	0.019
Carbohydrate degradation (polysaccharides)	0.012	0.012	0.012	0.010
Vitamin K production	0.002	0.002	0.002	0.009
Inflammation indicators	0.003	0.004	0.004	0.005
Gut barrier function	0.003	0.003	0.002	0.004
Cytotoxins	0.002	0.002	0.002	0.003
Constipation indicators	0.004	0.002	0.002	0.002
Lactose intolerance	0.000	0.000	0.000	0.000
Fat degradation	0.000	0.000	0.000	0.000
Sleep and the state of mind	0.000	0.000	0.000	0.000

represented in modules such as ‘energy metabolism and hyperacidity’, ‘fructose intolerance’, ‘vitamin K production’, ‘inflammation indicators’, ‘intestinal barrier function’, and ‘cytotoxins’ but not in the signaling pathways belonging to the carbohydrate degradation modules. In certain modules, no differences were found between the values of carnivores and omnivores, not surprisingly in the ‘protein fermentation’ module.

Based on our results, the gut microbiome of the carnivore showed few differences from the microbiome of the control group. The diversity and richness were comparable, and albeit differences were seen regarding its composition, the functionality in the context of functional modules did not differ by great means.

Although some different taxa were detected and the functionality of the microbiome was slightly impaired in terms of carbohydrate degradation, the detected differences were lower than expected. It is safe to say that the differences in the carnivore’s gut microbiome were far less drastic than the dietary restrictions he had imposed on himself deliberately. Although maybe not as balanced as desired on the phyla level, the carnivore’s gut microbiome is relatively diverse and displays some of the most important metabolic functions, indicating a desirable level of gut microbiome functionality. This is confirmed by the subject’s good general health, which is the most important desired outcome of any dietary intervention. Although a high-protein diet is often linked to impairment of renal function, the gut microbiome of the study subject does not bear taxonomic and functional traits characteristic for renal patients (Lohia, Vlahou & Zoidakis 2022, Wang *et al.* 2023). One evident problem in the gut microbiome composition is the increased abundance of genera *Escherichia* and *Salmonella*. Although both genera encompass a variety of bacterial species which behave differently in the gut, some of them are considered potentially pathogenic, especially *Salmonella*, that may contribute to chronic inflammation and carcinogenesis in host physiology (Zha, Garrett & Sun 2019, Rogers, Tsolis & Bäumler 2021).

Considering the growing trend of ketogenic dietary regimes such as the carnivore diet, there is a growing fear among health professionals that the absence of dietary fiber and microbiota-accessible carbohydrates would severely impair the composition and functionality of the gut microbiome (Ojo *et al.* 2021).

The growing interest in the ketogenic diet is linked with reduced ingestion of dietary fiber that is considered very important for gut composition and functionality (Zeng *et al.* 2022). Our study indicates that adherence to a carnivorous diet does not cause detrimental changes in the gut microbiome. Instead, it suggests that the effects on the gut microbiome are due to the combined influences of dietary regime and lifestyle, rather than meat consumption alone.

Further research is needed to identify which components of the carnivore diet could act as prebiotics in the absence of plant-derived prebiotics and maintain gut health over time.

It is important to see whether the presence of potential pathogens in great numbers is a systemic consequence of the carnivore diet or an artefact particular to the subject.

There are still many open questions in the field of the gut microbiome. Our study, it seems, has opened even more new ones. We still do not know what a healthy gut microbiome is and cannot say whose gut microbiome in the present study is the healthiest: the carnivore’s, the regular meat eater’s, or vegan’s? We do not have the data to conclude whether the carnivore’s gut microbiome adapted well to his dietary choices, what would be proof of the presumed high plasticity of the gut microbiome

(Thriene & Michels 2023) or whether the carnivore developed a diverse and functional gut microbiome by forcing his dietary choices upon the gut microbiome and therefore applying selective pressure (Ecklu-Mensah, Gilbert & Devkota 2022)? Although this study cannot answer these questions, it could demonstrate that the interaction between diet and the gut microbiome is more complex than expected and that there is no one-size-fits-all nutrition for the gut microbiome.

Limitations

This study, based on the diet of a single carnivore individual and a single measurement point, has significant limitations and should not be generalized; future research should include gut microbiome samples from a larger number of healthy carnivores and multiple stool samples to account for variations due to circadian rhythms, seasonal changes, and environmental factors.

Conclusions

Based on our study, we can conclude that it is possible to maintain a relatively diverse and functional gut microbiome on dietary extremes such as the carnivore diet. The particularities of the carnivore's gut microbiome regarding its composition and functionality are not attributable solely to meat consumption. The potential of non-prototypical prebiotic compounds diet has to be explored further.

Supplementary materials
This is linked to the online version of the paper at https://doi.org/10.1530/MAH-24-0006.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the study reported.

Funding
This work did not receive any specific grant from any funding agency in the public, commercial, or not-for-profit sector.

Institutional Review Board Statement
The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of the Gut Microbiome Center.

Informed Consent Statement
Informed consent was obtained from all subjects involved in the study.

Data Availability Statement
Data not made available in the the supplementary material can be accessed upon request to the corresponding author.

Author contribution statement
Conceptualization, AK and PD; Methodology, PD and ID.; Software, ID; Validation, IR, AL and ID; Formal Analysis, ID; Investigation, PXD; Resources, AK; Data Curation, ID; Writing – Original Draft Preparation, AK; Writing – Review and Editing, ID; AL; and PD; Visualization, AK; Supervision, AL; Project Administration, IR.

Acknowledgement
We thank the participant MS.

References

Basciani S, Camajani E, Contini S, *et al.* 2020 Very-low-calorie ketogenic diets with whey, vegetable, or animal protein in patients with obesity: a randomized pilot study.. *Journal of Clinical Endocrinology and Metabolism* **105**. (https://doi.org/10.1210/clinem/dgaa336)

Capling L, Gifford JA, Beck KL, *et al.* 2019 Development of an athlete diet index for rapid dietary assessment of athletes. *International Journal of Sport Nutrition and Exercise Metabolism* **29** 643–650. (https://doi.org/10.1123/ijsnem.2019-0098)

Caspi R, Billington R, Ferrer L, *et al.* 2016 The MetaCyc database of metabolic pathways and enzymes and the BioCyc collection of pathway/genome databases. *Nucleic Acids Research* **44** D471–D480. (https://doi.org/10.1093/nar/gkv1164)

Caspi R, Billington R, Keseler IM, *et al.* 2020 The MetaCyc database of metabolic pathways and enzymes - a 2019 update. *Nucleic Acids Research* **48** D445–D453. (https://doi.org/10.1093/nar/gkz862)

Chassaing B, Compher C, Bonhomme B, *et al.* 2022 Randomized controlled-feeding study of dietary emulsifier carboxymethylcellulose reveals detrimental impacts on the gut microbiota and metabolome. *Gastroenterology* **162** 743–756. (https://doi.org/10.1053/j.gastro.2021.11.006)

Cho CE, Taesuwan S, Malysheva OV, *et al.* 2017 Trimethylamine-N-oxide (TMAO) response to animal source foods varies among healthy young men and is influenced by their gut microbiota composition: a randomized controlled trial. *Molecular Nutrition & Food Research* **61**. (https://doi.org/10.1002/mnfr.201600324)

David LA, Maurice CF, Carmody RN, *et al.* 2014 Diet rapidly and reproducibly alters the human gut microbiome. *Nature* **505** 559–563. (https://doi.org/10.1038/nature12820)

Devi N, Madaan P, Kandoth N, *et al.* 2023 Efficacy and safety of dietary therapies for childhood drug-resistant epilepsy: a systematic review and network meta-analysis. *JAMA Pediatrics* **177** 258–266. (https://doi.org/10.1001/jamapediatrics.2022.5648)

Dhakal S, Moazzami Z, Perry C, *et al.* 2022 Effects of lean pork on microbiota and microbial-metabolite trimethylamine-N-oxide: a randomized controlled non-inferiority feeding trial based on the dietary guidelines for Americans. *Molecular Nutrition & Food Research* **66** e2101136. (https://doi.org/10.1002/mnfr.202101136)

Douglas GM, Maffei VJ, Zaneveld JR, *et al.* 2020 PICRUSt2 for prediction of metagenome functions. *Nature Biotechnology* **38** 685–688. (https://doi.org/10.1038/s41587-020-0548-6)

Ecklu-Mensah G, Gilbert J & Devkota S 2022 Dietary selection pressures and their Impact on the gut microbiome.. *Cellular and Molecular Gastroenterology and Hepatology* **13** 7–18. (https://doi.org/10.1016/j.jcmgh.2021.07.009)

Foster GD, Wyatt HR, Hill JO, *et al.* 2003 A randomized trial of a low-carbohydrate diet for obesity. *New England Journal of Medicine* **348** 2082–2090. (https://doi.org/10.1056/NEJMoa022207)

García-Conesa M-T, Philippou E, Pafilas C, *et al.* 2020 Exploring the validity of the 14-Item mediterranean diet adherence screener (MEDAS): a cross-national study in seven european countries around the mediterranean region. *Nutrients* **12**. (https://doi.org/10.3390/nu12102960)

Ghosh TS, Rampelli S, Jeffery IB, *et al.* 2020 Mediterranean diet intervention alters the gut microbiome in older people reducing frailty and improving health status: the NU-AGE 1-year dietary intervention across five European countries. *Gut* **69** 1218–1228. (https://doi.org/10.1136/gutjnl-2019-319654)

Guthrie L, *et al.* 2022 Impact of a 7-day homogeneous diet on interpersonal variation in human gut microbiomes and metabolomes. *Cell host & microbe* **30** 863-874.e4. (https://doi.org/10.1016/j.chom.2022.05.003)

Human Microbiome Project Consortium 2012*a* A framework for human microbiome research. *Nature* **486** 215–221. (https://doi.org/10.1038/nature11209)

Human Microbiome Project Consortium 2012*b* Structure, function and diversity of the healthy human microbiome. *Nature* **486** 207–214. (https://doi.org/10.1038/nature11234)

Johnston BC, Kanters S, Bandayrel K, *et al.* 2014 Comparison of weight loss among named diet programs in overweight and obese adults: a meta-analysis. *JAMA* **312** 923–933. (https://doi.org/10.1001/jama.2014.10397)

Koeth RA, Lam-Galvez BR, Kirsop J, *et al.* 2019 l-carnitine in omnivorous diets induces an atherogenic gut microbial pathway in humans. *Journal of Clinical Investigation* **129** 373–387. (https://doi.org/10.1172/JCI94601)

Lennerz BS, Mey JT, Henn OH, *et al.* 2021 Behavioral characteristics and self-reported health status among 2029 adults consuming a 'carnivore diet'. *Current Developments in Nutrition* **5** nzab133. (https://doi.org/10.1093/cdn/nzab133)

Lohia S, Vlahou A & Zoidakis J 2022 Microbiome in chronic kidney disease (CKD): an omics perspective. *Toxins* **14**. (https://doi.org/10.3390/toxins14030176)

Manral M, Dwivedi R, Gulati S, *et al.* 2023 Safety, efficacy, and tolerability of modified atkins diet in persons with drug-resistant epilepsy: a randomized controlled trial. *Neurology* **100** e1376–e1385. (https://doi.org/10.1212/WNL.0000000000206776)

Martin-McGill KJ, Bresnahan R, Levy RG, *et al.* Ketogenic diets for drug-resistant epilepsy. *Cochrane Database of Systematic Reviews* **6** CD001903. (https://doi.org/10.1002/14651858.CD001903.pub5)

McKay AKA, Stellingwerff T, Smith ES, *et al.* 2022 Defining training and performance caliber: a participant classification framework. *International Journal of Sports Physiology and Performance* **17** 317–331. (https://doi.org/10.1123/ijspp.2021-0451)

Meslier V, Laiola M, Roager HM, *et al.* 2020 Mediterranean diet intervention in overweight and obese subjects lowers plasma cholesterol and causes changes in the gut microbiome and metabolome independently of energy intake. *Gut* **69** 1258–1268. (https://doi.org/10.1136/gutjnl-2019-320438)

Ojo O, Ojo OO, Zand N, *et al.* 2021 The effect of dietary fibre on gut microbiota, lipid profile, and inflammatory markers in patients with type 2 diabetes: a systematic review and meta-analysis of randomised controlled trials. *Nutrients* **13**. (https://doi.org/10.3390/nu13061805)

Pfeil J, Siptroth J, Pospisil H, *et al.* 2023 Classification of microbiome data from type 2 diabetes mellitus individuals with deep learning image recognition. *Big Data and Cognitive Computing* **7**. (https://doi.org/10.3390/bdcc7010051)

Rogers AWL, Tsolis RM & Bäumler AJ 2021 Salmonella versus the microbiome. *Microbiology and Molecular Biology Reviews* **85**. (https://doi.org/10.1128/MMBR.00027-19)

Sellem L, Srour B, Javaux G, *et al.* 2024 Food additive emulsifiers and cancer risk: results from the french prospective NutriNet-Santé cohort. *PLoS Medicine* **21** e1004338. (https://doi.org/10.1371/journal.pmed.1004338)

Shen W, Wolf PG, Carbonero F, *et al.* 2014 Intestinal and systemic inflammatory responses are positively associated with sulfidogenic bacteria abundance in high-fat-fed male C57BL/6J mice.. *Journal of Nutrition* **144** 1181–1187. (https://doi.org/10.3945/jn.114.194332)

Siptroth J, Moskalenko O, Krumbiegel C, *et al.* 2023*a* Investigation of metabolic pathways from gut microbiome analyses regarding type 2 diabetes mellitus using artificial neural networks. *Discover Artificial Intelligence* **3** 19. (https://doi.org/10.1007/s44163-023-00064-6)

Siptroth J, Moskalenko O, Krumbiegel C, *et al.* 2023*b* Variation of butyrate production in the gut microbiome in type 2 diabetes patients. *International Microbiology* **26** 601–610. (https://doi.org/10.1007/s10123-023-00324-6)

Sondhi V, Agarwala A, Pandey RM, *et al.* 2020 Efficacy of ketogenic diet, modified atkins diet, and low glycemic index therapy diet among children with drug-resistant epilepsy: a randomized clinical trial. *JAMA Pediatrics* **174** 944–951. (https://doi.org/10.1001/jamapediatrics.2020.2282)

Teigen L, Mathai PP, Lopez S, *et al.* 2022 Differential hydrogen sulfide production by a human cohort in response to animal- and plant-based diet interventions. *Clinical Nutrition* **41** 1153–1162. (https://doi.org/10.1016/j.clnu.2022.03.028)

Thriene K & Michels KB 2023 Human gut microbiota plasticity throughout the life course. *International Journal of Environmental Research and Public Health* **20**. (https://doi.org/10.3390/ijerph20021463)

Van den Houte K, Colomier E, Routhiaux K, *et al.* 2024 Efficacy and findings of a blinded randomized reintroduction phase for the low FODMAP diet in irritable bowel syndrome. *Gastroenterology* **167** 333–342. (https://doi.org/10.1053/j.gastro.2024.02.008)

Wang H, Ainiwaer A, Song Y, *et al.* 2023 Perturbed gut microbiome and fecal and serum metabolomes are associated with chronic kidney disease severity. *Microbiome* **11** 3. (https://doi.org/10.1186/s40168-022-01443-4)

Whelan K, Bancil AS, Lindsay JO, *et al.* 2024 Ultra-processed foods and food additives in gut health and disease. *Nature Reviews. Gastroenterology and Hepatology* **21** 406–427. (https://doi.org/10.1038/s41575-024-00893-5)

Wolf PG, Gaskins HR, Ridlon JM, *et al.* 2020 Effects of taurocholic acid metabolism by gut bacteria: a controlled feeding trial in adult African American subjects at elevated risk for colorectal cancer. *Contemporary Clinical Trials Communications* **19** 100611. (https://doi.org/10.1016/j.conctc.2020.100611)

Wu GD, Chen J, Hoffmann C, *et al.* 2011 Linking long-term dietary patterns with gut microbial enterotypes. *Science* **334** 105–108. (https://doi.org/10.1126/science.1208344)

Wu W-K, Panyod S, Liu P-Y, *et al.* 2020 Characterization of TMAO productivity from carnitine challenge facilitates personalized nutrition and microbiome signatures discovery. *Microbiome* **8** 162. (https://doi.org/10.1186/s40168-020-00912-y)

Zeng X, Xing X, Gupta M, *et al.* 2022 Gut bacterial nutrient preferences quantified in vivo. *Cell* **185** 3441–3456.e19. (https://doi.org/10.1016/j.cell.2022.07.020)

Zha L, Garrett S & Sun J 2019 Salmonella infection in chronic inflammation and gastrointestinal cancer. *Diseases* **7**. (https://doi.org/10.3390/diseases7010028)

Zhu C, Sawrey-Kubicek L, Beals E, *et al.* 2020 Human gut microbiome composition and tryptophan metabolites were changed differently by fast food and Mediterranean diet in 4 days: a pilot study. *Nutrition Research* **77** 62–72. (https://doi.org/10.1016/j.nutres.2020.03.005)