Gut Microbiome (2026), **7**, e16, 1–22
doi:10.1017/gmb.2026.10033

GUTMICROBIOME

RESEARCH ARTICLE

Diet-derived microbial metabolites modulate stress-responsive gene expression in germ-free zebrafish

Jayson Dale R. Capistrano[1,2], Bianka Ketheeswaranathan[1,2], Matthew S. Horn[2], Pham Ngoc Giao Tran[2], Taylor Ball[2], Shalvi Chirmade[2], Sarah J. Vancuren[2], David W.L. Ma[3], Kathryn Walton[4], Emma Allen-Vercoe[2] and Terence J. Van Raay[1,2]

[1]Department of Molecular and Cellular Biology and Collaborative Neuroscience Program, University of Guelph, Canada; [2]Department of Molecular and Cellular Biology, University of Guelph, Canada; [3]Department of Human Health Science, University of Guelph, Canada and [4]Department of Family Relations and Applied Nutrition, University of Guelph, Canada
Corresponding author: Terence Van Raay; Email: tvanraay@uoguelph.ca

(Received 26 May 2026 UTC; revised 04 September 2026 UTC; accepted 01 October 2026 UTC)

Abstract

The gut microbiome plays a pivotal role in overall host health, yet the extent at which diet-derived microbial metabolites affect neurodevelopment and inflammation remains unclear. Here, we employed the *robogut* bioreactor system seeded with faecal samples from two healthy paediatric donors to generate microbial communities exposed to four different diets: low-fibre Western, high-fibre Western, Mediterranean, and Yanomami, as well as three fibre supplements: fruit and vegetable fibre, cereal fibre, and resistant starch fibre. Metabolites produced by these microbial communities were isolated and applied to germ-free zebrafish (*Danio rerio*) embryos to assess their effects on neurodevelopment and inflammatory gene expression under basal and stress-induced conditions. Despite minimal changes in microbial composition across diets and fibre sources, significant differences in short-chain fatty acid concentrations were observed. , Under basal conditions, metabolite treatments had limited effects on the expression of neural and inflammatory genes. Under stress conditions, metabolites from any diet mitigated stress-induced *bdnf* expression, suggesting a possible modulatory role of microbial metabolites on stress responses. Overall, these findings highlight the resilience of microbial communities to dietary changes and underscore the importance of microbial metabolite output and its donor-specific nature in influencing host neurodevelopment and immune responses.

Keywords: gut microbiome; microbial metabolites; dietary fibre; inflammatory gene expression; stress response; robogut bioreactor; short-chain fatty acids (SCFAs); germ free zebrafish model

Introduction

The importance of the gut microbiome in maintaining host health and physiology has been well-established in the literature (Cryan and Dinan, 2012; McFall-Ngai et al., 2013; Luczynski et al., 2016; Wu et al., 2016; Van de Wouw et al., 2018; Biesiekierski et al., 2019; Cryan et al., 2019; Leeming et al., 2019; Lavelle and Sokol, 2020; Hamamah and Covasa, 2022; Pate et al., 2023; Delgado-Ocaña and Cuesta, 2024; O'Riordan et al., 2025). It is comprised of microorganisms that reside within the host gut and encompasses their complete genetic and metabolic functions (Barko et al., 2018; Silva et al., 2020; Sabit et al., 2021). Its overall composition depends on both intrinsic and extrinsic factors (Cho and Blaser, 2012; Wen and Duffy, 2017; Cryan, 2025) with diet emerging as a strong predictor in determining gut microbiome composition and diversity (Wu et al., 2011; Moles and Otaegui, 2020; Redondo-Useros et al., 2020; Berding et al., 2021). Specifically, several studies have shown that consumption of a high-fat

© The Author(s), 2026. Published by Cambridge University Press in association with The Nutrition Society. This is an Open Access article, distributed under the terms of the Creative Commons Attribution licence (http://creativecommons.org/licenses/by/4.0), which permits unrestricted re-use, distribution and reproduction, provided the original article is properly cited.

Western diet increased the Bacillota/Bacteroidota ratio (Cani et al., 2007; Ohland et al., 2013; Patterson et al., 2014; Jørgensen et al., 2015; Velázquez et al., 2019; Malesza et al., 2021; Marx et al., 2021) and reduced *Bifidobacterium* spp. (Cani et al., 2007; Marx et al., 2021; Schneider et al., 2024), while prebiotic supplementation increased the ratio of beneficial microbes (Van de Wouw et al., 2018; Marx et al., 2021; Rosell-Cardona et al., 2025).

Epidemiological studies have further demonstrated that certain diets can have beneficial effects, modulate levels of microbial metabolites, and prevent the onset of diseases (Gill et al., 2021; Horn et al., 2022; O'Riordan et al., 2022; Heslin and McNulty, 2023; Ross et al., 2024; Schneider et al., 2024; Cryan, 2025). For example, diets rich in fibre and legumes (as in the Mediterranean diet) have been linked to reductions in the onset of chronic disease, anti-inflammatory effects, and better mental health (De Filippis et al., 2016; Lassale et al., 2019; Asnicar et al., 2021; Jennings et al., 2024; Rostgaard-Hansen et al., 2024; Schneider et al., 2024), whereas low-fibre, high-fat, and high-sugar diets have been associated with an increased risk of cardiovascular disease, obesity, and depression (Li et al., 2017; Duan et al., 2018; Lassale et al., 2019; Wali et al., 2020; Wang et al., 2020; Collins et al., 2022; Zeng et al., 2023; Schneider et al., 2024). Expectedly, perturbations in the gut microbiome are often reported in diseased states (Chen et al., 2021; De Vos et al., 2022; Liu et al., 2022; Gan et al., 2024), which emphasises the involvement of diets in shaping the gut microbiome.

The gut microbiome breaks down indigestible fibres from diets via fermentation (Mayer et al., 2015; San Mauro Martin et al., 2018; Liu et al., 2022; Aleman et al., 2023) to produce metabolites (O'Riordan et al., 2022; Rosell-Cardona et al., 2025) that are predominantly made up of short-chain fatty acids (SCFAs) (Kim, 2023) that can enter systemic circulation, cross the blood–brain barrier, and ultimately affect the brain (Cryan and Dinan, 2012; Mayer et al., 2015; Agustí et al., 2018; Appleton, 2018; Loh et al., 2024). However, the exact mechanisms and contributions of these metabolites remain elusive. In previous studies, SCFA treatments were reported to alleviate stress, ameliorate depressive behaviours, increase the growth rate of neural cells, and improve memory (Cryan and Dinan, 2012; Mayer et al., 2015; Agustí et al., 2018; Appleton, 2018; Van de Wouw et al., 2018; Cryan et al., 2019; Marx et al., 2021; Horn et al., 2022; Loh et al., 2024; Ross et al., 2024; Schneider et al., 2024; Cryan, 2025; O'Riordan et al., 2025; Rosell-Cardona et al., 2025). Although these studies emphasise the neuroactive effects of metabolites, many of these treatments were limited to individual SCFAs such as sodium butyrate. The gut microbiome, however, produces numerous metabolites (Topping and Clifton, 2001; Mayer et al., 2015; Postler and Ghosh, 2017; San Mauro Martin et al., 2018; Liu et al., 2022) which likely work in concert. Thus, there is an absence of empirical evidence that illustrates how different diets affect the nervous system.

In this study, we employed different diets and fibre sources based on their well-documented effects on the gut microbiome and association with overall health. The low-fibre Western (LFW) diet includes foods that are highly processed and contain high amounts of added sugars, fats, and salt. This diet is associated with low consumption of fibre and has been linked to many high-risk diseases and disorders, including obesity, diabetes, anxiety, and depression (Li et al., 2017; Statovci et al., 2017; Duan et al., 2018; Lassale et al., 2019; Wali et al., 2020; Wang et al., 2020; Shi et al., 2021; Collins et al., 2022; Zeng et al., 2023; Schneider et al., 2024). In contrast, the high-fibre Western (HFW) diet follows a largely plant-based diet that often excludes food products of animal origins (Orlich et al., 2013; Xiao et al., 2022), while the Mediterranean (MED) diet is similar but involves the consumption of fish and poultry products (Davis et al., 2015; De Filippis et al., 2016; Lassale et al., 2019; Ventriglio et al., 2020; Asnicar et al., 2021; Jennings et al., 2024; Rostgaard-Hansen et al., 2024; Schneider et al., 2024). Both diets have high fibre contents which is beneficial to the gut microbiome and are linked to better overall health (Orlich et al., 2013; Davis et al., 2015; De Filippis et al., 2016; Lassale et al., 2019; Ventriglio et al., 2020; Asnicar et al., 2021; Xiao et al., 2022; Jennings et al., 2024; Rostgaard-Hansen et al., 2024; Schneider et al., 2024). Finally, the Yanomami (YAN) diet is modelled on the substrate consumption of the Yanomami people of the Amazon, many of whom still subside on fishing, gathering, hunting, and simple horticulture (Mueller et al., 2018; Conteville et al., 2019). Their diets are remarkably low in salt and fat content and are extremely high in fibre (Mueller et al., 2018; Conteville et al., 2019), and those Yanomami who still follow traditional subsistence patterns tend to show very high levels of cardiometabolic health and low

incidence of chronic diseases (Deehan et al., 2017; Mueller et al., 2018; Conteville et al., 2019), which warrants further investigation. In addition, we also explored the role of fibres by supplementing the LFW diet with soluble and insoluble fibres due to their known benefits and role in modulating the gut microbiome (Deehan et al., 2017; Cronin et al., 2021). Soluble fibres can be readily metabolised by gut microbes and, therefore, influence the abundance and diversity of the gut microbiota. On the other hand, insoluble fibres are poorly metabolised by gut microbes and instead play a role in bulking waste material in the gut, increasing the transit rate throughout the gut, and potentially decreasing the time available for gut microbes to ferment non-digested food particles (Koh et al., 2016; Staller et al., 2018; Gill et al., 2021; Guan et al., 2021).

Given the relationship between diet, fibre, and health, we endeavoured to test the effects of metabolites sourced from different diets and fibres on gene expression in an animal model. We hypothesised that metabolites derived from high-fibre diets would have a positive effect on neurodevelopment and inflammatory gene expression. To test this, we first generated metabolites using a bioreactor that mimics the distal colon to model the different diets and fibre sources and then used these metabolites to treat our germ-free zebrafish model, which we previously demonstrated requires microbial metabolites for a proper nervous system development (Rea et al., 2022; Gianetto-Hill et al., 2023).

Methods

Collection of faecal donor samples

Faecal samples from 10 donors (5 male, 5 female), aged 2 to 6 years old, with a low history of antibiotic use and clinical illnesses and who followed a typical Western diet were evaluated. The samples were collected from participants of the Guelph Family Health Study in accordance with the University of Guelph's research ethics board approval (REB# 17-07-003) and stored at −80 °C until use. Written informed consent was obtained from each participant or their appropriate guardian.

Shotgun metagenomic sequencing

Faecal DNA was isolated using the QIAamp® PowerFecal® Pro DNA Kit (QIAGEN) and subjected to whole metagenomic shotgun sequencing using Illumina NextSeq 2000 at the University of Pittsburgh's Microbial Genome Sequencing Center, generating 2x151bp paired-end reads with an average of 7,804,105 forward and reverse reads from all samples. Sequencing quality was evaluated using FastQC v.11.9 (Babraham Bioinformatics, 2010). Sequences that were less than 50 bp in length were removed using Trimmomatic v.0.39 (Bolger et al., 2014). Forward and reverse reads were joined using Fastq-join v.1.3.1 (Babraham Bioinformatics, 2010). Relative abundance of the microbial taxa was obtained using MetaPhlAn 3 with the ChocoPhlAn 3 database (Beghini et al., 2021). Bowtie2 was used to align the sequences to clade-specific marker genes (Langmead and Salzberg, 2012). Analyses and visualisation on the resulting microbial composition data were carried out in R v.4.1.2.

Expansion of faecal samples

To provide enough biomass for all diet and fibre experiments, the microbial communities from the selected faecal samples were subsequently expanded using bioreactors (Multifors, Switzerland) under batch conditions for 3 days (McDonald et al., 2013). Approximately 3.00 g of the selected faecal samples from donors 1 and 2 were used to seed (0.5% w/v) independent bioreactor systems supplied with the HFW diet growth media (McDonald et al., 2013). Each faecal sample was expanded twice, yielding approximately 1 L of biomass, which was divided into 20 mL aliquots and stored in −80 °C to act as starting inocula for further bioreactor experiments.

Bioreactor set-up, sampling, and maintenance

The bioreactor experiment followed the protocol outlined by Gianetto-Hill et al. (2023). Growing conditions mimicked the environment of the human distal colon (37 °C, pH 7.0, gentle agitation, and under anaerobic conditions from constant sparging with N_2 gas) and were inoculated with 10% v/v of the expansion culture and allowed to grow in the diet growth media for 24 hours (Day 0). On Day 1, media flow was started with the diet growth media at a rate to match a 24-hour retention time. Overall, both faecal donor samples were exposed to 4 different diets and 3 different fibre sources, with a total of 2 replicates per faecal donors. Formulations of the various diet growth media used are detailed in Table 1 (Macfarlane et al., 1998; McDonald et al., 2013). The diet experiments lasted for 21 days and were supplied with either a LFW, HFW, MED, or YAN diet (Figure 1). The LFW diet contains approximately 19 g of fibre, while the HFW diet contains 29 g. The MED diet has approximately 19 g of fibre but also contains legume powder, polyphenols, vegetable peptone, and fish hydrolysate. Finally, the YAN diet contains approximately 33 g of fibre and receives a daily supplementation of 1 g of fibre from plantain flour and cellulose. For the fibre experiments, the bioreactors were supplied with the LFW diet until day 17 and were subsequently provided with supplements of either fruit and vegetable fibre (FVF; 10 g of fibre from pectin), resistant starch (RSF; 9.0 g of fibre from corn starch), cereal fibre (CRF; 9.0 g of fibre from oat fibre and wheat bran) or left with the LFW diet until Day 28 (Figure 1). The bioreactors were sampled in triplicates on Days 0, 1, and 2, then on alternating days and were stored in −80 °C. At the conclusion of each experiment, the vessel contents were harvested, aliquoted, and stored in −80 °C.

Extraction of bioreactor metabolites

Cell-free metabolites were isolated from the vessel contents of the bioreactor harvested on the last day of each bioreactor experiment (adapted from Ganobis et al., 2020). In brief, frozen effluents were thawed at sequentially lower temperatures, first from −80 °C to −20 °C for at least 12 hours and then −20 °C to 4 °C for at least 12 hours. Once thawed, the samples were centrifuged at 5,000 × g for 15 minutes at 4 °C to remove crude particulate matter and then again at 14,000 × g for 15 minutes at 4 °C to remove cells and other remaining particulates. The resulting supernatant was filter-sterilised using a 0.22 μM polyether sulfone filter and stored at −20 °C until further processing. Every metabolite sample was plated on a non-selective brain-heart infusion (BHI) plate and incubated at 28.5 °C for 24 hours to confirm sterility (i.e., zero visible colonies).

16S rRNA gene sequencing

Genomic DNA was extracted using the QIAmp® PowerFecal® Pro DNA Kit (QIAGEN) following the manufacturer's instructions. Partial 16S ribosomal RNA (rRNA) gene sequences of the V3–V4 regions were amplified via PCR and sequenced on the Illumina MiSeq platform (2 × 300 bp, at a depth of 100,000 reads per sample) at the University of Guelph's Advanced Analysis Centre. The R package DADA2 v1.30.0 was used to process paired end reads and create amplicon sequence variant (ASV) tables. Taxonomy was assigned using the *assignTaxonomy* function from the DADA2 package with a set of reference sequences via the Silva 138.1 prokaryotic SSU database. Resulting classified ASV data were imported to Phyloseq v1.38.0 for visualisation.

Nuclear magnetic resonance (NMR) spectroscopy and analysis

To determine the metabolic profiles of the bioreactor samples, prepared metabolites were mixed with 10% Chenomx IS-2 standard containing 4.64 mM 3-(Trimethylsilyl)-1-propanesulphonic acid sodium salt in deuterated water and subjected to 1-D ^{1}H NMR using the Bruker Avance 600 MHz NMR spectrometer at the University of Guelph NMR Facility. The concentrations of major SCFAs in the NMR spectra were determined via the Chenomx NMR Suite program, version 8.6.

Table 1. Formulations for each diet growth media

	Diet Experiment				Fibre Experiment		
Component (g/2L)	Low Fibre (LFW)	High Fibre (HFW)	Mediterranean (MED)	Yanomami (YAN)	Fruit and Vegetable Fibre (FVF)	Resistant Starch (RSF)	Cereal Fibre (CRF)
Arabinogalactan	1.00	4.00	4.00	4.00	1.00	1.00	1.00
Bacto™ Yeast Extract	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Bile Salts	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Calcium Chloride ($CaCl_2$)	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Dipotassium Phosphate (K_2HPO_4)	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Casein	6.00	6.00	–	–	6.00	6.00	6.00
Cassava Flour	–	–	–	16.80	–	–	–
Cellulose	–	–	–	0.50/day	–	–	–
Corn Starch	3.00	3.00	3.00	–	3.00	9.00	3.00
Fish Hydrolysate	–	–	4.00	4.00	–	–	–
Hemin	2.00 mL	2.00 mL	2.00 mL	2.00 mL	2.00 mL	2.00 mL	2.00 mL
Inulin	1.00	2.00	3.00	2.00	1.00	1.00	1.00
L-Cysteine HCl	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Legume Protein	–	–	4.00	–	–	–	–
Magnesium Sulphate ($MgSO_4$)	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Menadione	2.00 mL	2.00 mL	2.00 mL	2.00 mL	2.00 mL	2.00 mL	2.00 mL
Mucin	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Mushroom Powder	–	–	–	2.00	–	–	–
Oat Fibre	–	–	–	–	–	–	4.50
Palmetto	–	–	–	0.50/day	–	–	–
Pectin	1.00	4.00	4.00	4.00	10.00	1.00	1.00
Peptone Water	4.00	4.00	–	–	4.00	4.00	4.00
Plantain Flour	–	–	–	0.50/day	–	–	–
Polyphenols	–	–	1.00	–	–	–	–
Potassium Dihydrogen (KH_2PO_4)	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Potato Starch	3.00	3.00	–	–	3.00	3.00	3.00
Sodium Bicarbonate ($NaHCO_3$)	4.00	4.00	4.00	4.00	4.00	4.00	4.00

Table 1. *Continued*

Component (g/2L)	Diet Experiment				Fibre Experiment		
	Low Fibre (LFW)	High Fibre (HFW)	Mediterranean (MED)	Yanomami (YAN)	Fruit and Vegetable Fibre (FVF)	Resistant Starch (RSF)	Cereal Fibre (CRF)
Sodium Chloride (NaCl)	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Vegetable Peptone	–	–	4.00	1.80	–	–	–
Wheat Starch	3.00	3.00	–	–	3.00	3.00	3.00
Wheat Bran	–	–	–	–	–	–	4.50
Xylo-oligosaccharides	1.00	4.00	4.00	4.00	1.00	1.00	1.00

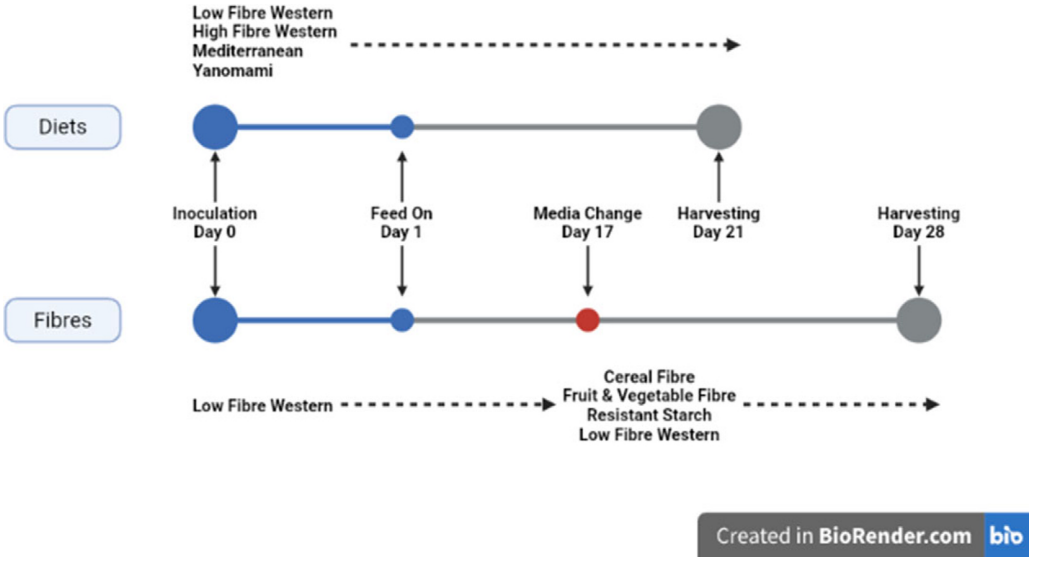


Figure 1. Flowchart of the bioreactor experiment. All bioreactors were inoculated with the expanded faecal samples on Day 0. Beginning on Day 1, the bioreactors received the diet media at a constant rate. The diet experiments received the same type of diet growth media until Day 21, while the fibre experiments all received the LFW diet until Day 28 or was switched to a different fibre source on Day 17 until Day 28. CRF, cereal fibre; FVF, fruit and vegetable fibre; HFW, high fibre Western; LFW, low fibre Western; MED, Mediterranean; RSF, resistant starch fibre; YAN, Yanomami.

Zebrafish maintenance and embryo spawning

Adult wild-type Tübingen (TU) zebrafish (*Danio rerio*) lines (1 to 1.5 years of age) were kept and maintained in 8 L aquaria in mixed sex groups in a dedicated zebrafish rack system (Tecniplast) with automated temperature, pH, and conductivity control in the Hagen Aqualab at the University of Guelph. The system was maintained at 28.5 °C, pH 7.0, 450–550 µS/cm on a 14:10 light/dark simulated photoperiod and fed commercial flake food or brine shrimp 2–3 times daily. Water chemistry was tested weekly and ammonia, nitrate, and nitrite conditions were routinely at 0.0 ppm.

Prior to the end of the photoperiod and preceding the day of embryo collection, adult male and female zebrafish were sexed and sorted into spawning baskets with a barricade to separate the sexes. At the onset

of the next photoperiod, the barricades were removed to allow natural spawning. Embryos were collected within 2 hours post-fertilisation (2hpf) and kept in zebrafish embryo medium (EM; 0.00006% w/v Instant Ocean® Sea Salt solution and 0.001% methylene blue in purified distilled water) at 28.5 °C.

Generation and treatment of germ-free zebrafish

To explore the effects of metabolites, zebrafish embryos from pooled clutches were sterilised to remove exogenous bacteria. In brief, at 30% to 50% epiboly (approximately 5hpf), embryos were divided into conventionally raised (CV) and sterilised groups. The CV embryos were kept at room temperature (RT) alongside the embryos being sterilised to ensure similar development progression. Using sterile technique, the embryos were immersed in filter-sterilised gentamicin (100 µg/mL) for 1 hour, followed by three 5-minute washes in 0.003% filter-sterilised hypochlorite solution and sterile EM performed in a sterile biosafety cabinet (adapted from Pham et al., 2008). Post-sterilisation, sterilised embryos were further divided into treatment groups receiving 1% v/v metabolite treatment or no metabolite treatment (germ-free; GF) (adapted from Rea et al., 2022), with each group having 30–35 embryos each. This concentration was determined to be the highest concentration that did not induce mortality or developmental abnormalities.

All embryos were then incubated at 28.5 C and kept at the same 14:10 light/dark photoperiod. At 1-day post-fertilisation (dpf), one embryo from each treatment group was homogenised in 50 µL of sterile EM. A 20 µL sample was plated on BHI agar plates and then incubated at 28.5 °C for 24 hours to test for sterility. Upon confirmation of sterility (i.e., zero visible colonies), embryos (including the CV embryos) were kept at 28.5 °C in the same media and harvested at 3dpf. Embryos were routinely monitored, with abnormal or dead embryos/larva removed aseptically.

Heat, osmotic, and swirling stressor challenges

Sterilised embryos were assigned to treatment groups as described above. At 3dpf, 10 zebrafish larvae from each treatment group (diets only) were exposed to one stressor challenge lasting 1 hour alongside a non-stressed counterpart where the larvae remained at RT. Therefore, for a single stressor, there were 6 treatment groups (CV, GF, LFW, HFW, MED, and YAN), each with 10 larvae receiving a stressor alongside a non-stressed counterpart.

For the heat stress, larvae were placed in a 50 mL falcon tube containing 20 mL pre-warmed EM and submerged in a 37 °C water bath (adapted from Lim and Bernier, 2022). For the osmotic stress, larvae were transferred into a 10 cm petri dish containing 20 mL of a 150 mM salt solution and incubated at RT (adapted from Cheng et al., 2022). Lastly, for the swirling stress, larvae were transferred into a 10 cm petri dish containing 20 mL EM, placed on a high-speed rotator (Stovall Life Sciences, Inc.) and swirled at approximately 5 rotations per second (adapted from Alsop and Vijayan, 2008). Shortly after the stressor, 5 larvae were harvested and macerated in 700 µL Trizol, flash frozen at −80 °C, and processed for qRT-PCR.

Quantitative RT-PCR (qRT-PCR)

Total RNA from a pool of five 3dpf larvae was extracted with Trizol following the recommendations in the GENEzol™ TriRNA Pure Kit (Geneaid Biotech Ltd.). qRT-PCR was performed on the CFX96 Touch Real-Time Detection System (Bio-Rad) using the Luna Universal One-Step qRT-PCR Kit (New England Biolabs). Primer efficiencies were measured using SYBR Green Master Mix for qPCR (ThermoFisher) and confirmed to be within 95% to 110% (Table 2) using standard curves. Each biological replicate for qRT-PCR included 3 technical replicates and was normalised to the housekeeping gene, *ef1a*. Gene expressions were calculated using the Livak method and expressed in fold changes.

Table 2. Details of primer sequences used in qRT-PCR.

Gene		Sequence	Length (bp)	Primer Efficiency (%)
bdnf	Forward	5′-AACTCCAAAGGATCCGCTCA–3′	20	98.02
	Reverse	5′-GCAGCTCTCATGCAACTGA–3′	19	
ef1a	Forward	5′-CTGGAGGCCAGCTCAAACAT–3′	20	95.54
	Reverse	5′-ATCAAGAAGAGTAGTACCGCTAGCATTAC–3′	29	
fosab	Forward	5′-TCAACCAGACTCAGGAGTTCAC–3′	22	107.00
	Reverse	5′-CAAGTCCGGGCATGAAGAGA–3′	20	
il-6	Forward	5′-TCAACTTCTCCAGCGTGATG–3′	20	101.63
	Reverse	5′-TCTTTCCCTCTTTTCCTCCTG–3′	21	
tnfα	Forward	5′-GCGCTTTTCTGAATCCTACG–3′	20	108.70
	Reverse	5′-TGCCCAGTCTGTCTCCTTCT–3′	20	

Statistical analyses

All data obtained were tested for normality and equal variances. Differences between the treatment groups were analysed by an analysis of variance (ANOVA) or by a *t*-test (as specified in each figure) with significance set at $p < 0.05$. Data sets that do not meet normality and variance assumptions were evaluated using non-parametric tests. All analyses were performed using GraphPad Prism version 10.0.0 for Windows. For the diets and fibre source comparisons, LFW was used as the control. For the zebrafish comparisons, the CV group was used as the control. For the stressor challenge, a non-stressed baseline was used as the control. In the figure legend, uppercase "*N*" refers to the number of biological replicates.

Results

Donor sample selection

Metagenomic shotgun sequencing of the 10 donor samples was used to determine the taxonomic profiles of each faecal sample (Figure 2A). A Principal Component Analysis (PCA) was used to illustrate the variations between donors so that representative samples could be selected. The PCA plot revealed 3 clusters, wherein PC1 and PC2 describe 25.83% and 17.88% of the variation, respectively (Figure 2B). A third PC describes an additional 13.60% variation, but no new clusters were formed. In addition, a k-medoid model plot revealed similar findings in that 3 clusters were formed. In both plots, donors 6 and 8 formed their own clusters, while the rest of the samples clustered together. From these analyses, donors 1 and 2 were chosen as representatives of the pool as these samples were at the centre of its cluster. Donor 1 was a female, 4.3 years old with a history of one antibiotic use (specifically, amoxicillin) and donor 2 was a male, 3 years old with two recorded prior antibiotic use (specifically, amoxicillin and ampicillin). The timing of the antibiotic use is unknown.

Diets and fibre source did not impact the microbiota cultured in bioreactors

To determine if different diets affected overall microbial profiles, we submitted samples from each diet/fibre source and donor for 16S rRNA gene sequencing analyses at the end of the bioreactor experiments. Despite being supplied different diets, it was revealed that both donors had similar microbial profiles with no clear shifts in microbial compositions or diversity after exposure to each diet (Figure 3A). The distribution of the microbial compositions was found to be consistent between technical replicates, with

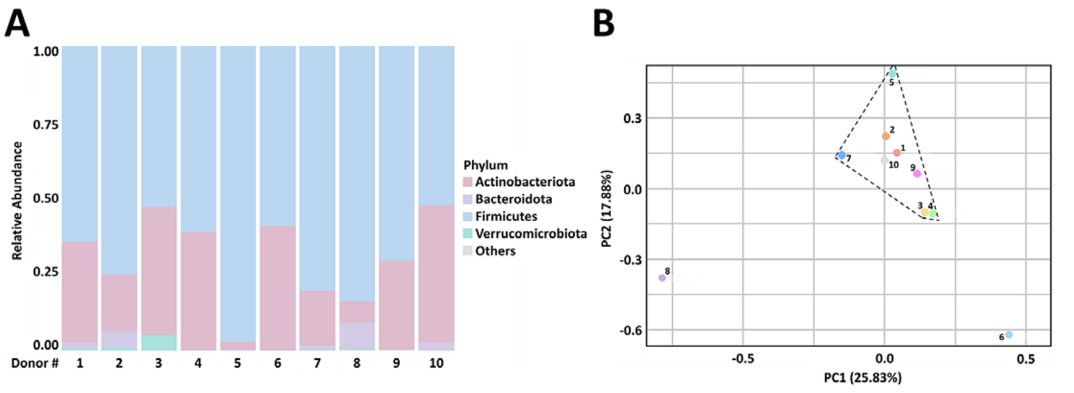


Figure 2. Microbial analysis of 10 donor samples. (A) Stacked bar plot showing the top four phyla detected in all 10 donors. The category "Other" describes the phyla with a maximum relative abundance of less than 5% from all samples and was only found in donors 1, 5, 7, 8, and 9. (B) Two-dimensional PCA plot showing the variance between all 10 donors. Enhanced cluster detection using k-medoid identification demonstrated that donors 6 and 8 are separate from the group. From this analysis, donors 1 and 2 were chosen to be the most representative of the group.

the most dominant phyla being Bacillota, Bacteroidota, and Verrucomicrobiota. A small portion of the microbes belongs to the Pseudomonadota and Actinomycetota phyla. We then sought to determine if different fibre sources influenced overall microbial profiles but saw no significant changes despite being supplied with different fibre sources (Figure 3B, Supplementary Figure S1). Collectively, this suggests that the different diets and the different fibre sources did not dramatically alter the microbial composition or diversity of the phyla over the course of the experiment.

A comparison of the diversity between the raw faecal samples (Figure 2) and the samples collected at the end of both bioreactor experiments (Figure 3) demonstrated that Bacillota remained one of the dominant phyla. We also observed a marked decrease in Actinomycetota and an increase in Bacteroidota after both experiments, suggesting that the media used in both experiments may have favoured the growth of the latter phyla over the former. Nonetheless, the microbial phyla seen at the end of both experiments are consistent with the microbes found in the human gut (Ley et al., 2006; Biagi et al., 2010; Arumugam et al., 2011; Tremaroli and Bäckhed, 2012; Vieira-Silva et al., 2016; Rinninella et al., 2019; Salazar et al., 2020; Malesza et al., 2021), indicating that the bioreactor is indeed a good model to mimic the environment of the gut.

Diets and fibre source have unique effects on microbial metabolite output

Despite the lack of appreciable microbial changes between diets or fibre sources, it is still possible that the metabolic profiles between the diets and/or fibre sources are different. To test this, we performed 1-D ^{1}H NMR to evaluate changes in the concentrations of the 3 major SCFAs: acetate, butyrate, and propionate. For the diet experiment, the SCFA concentrations remained relatively stable for the duration of the bioreactor runs (Figure 4A). Here, acetate concentrations were the highest across all diets, followed by propionate then butyrate concentrations; however, in the YAN diet, acetate concentrations were followed by butyrate instead of propionate concentrations. For the fibre experiment, steady increases in SCFA concentrations were observed following the addition of fibres at Day 17 (Figure 4B), with FVF having a meaningful effect on acetate levels. Overall, there were no significant differences in SCFA concentrations between donors (Supplementary Figures S2 and S3) and the changes observed over time were consistent between donors (*data not shown*).

We then compared the SCFA concentrations at the end of each experiment (Day 21 for the diets, Day 28 for the fibres) as these metabolites would be used for the subsequent experiments. Although we could

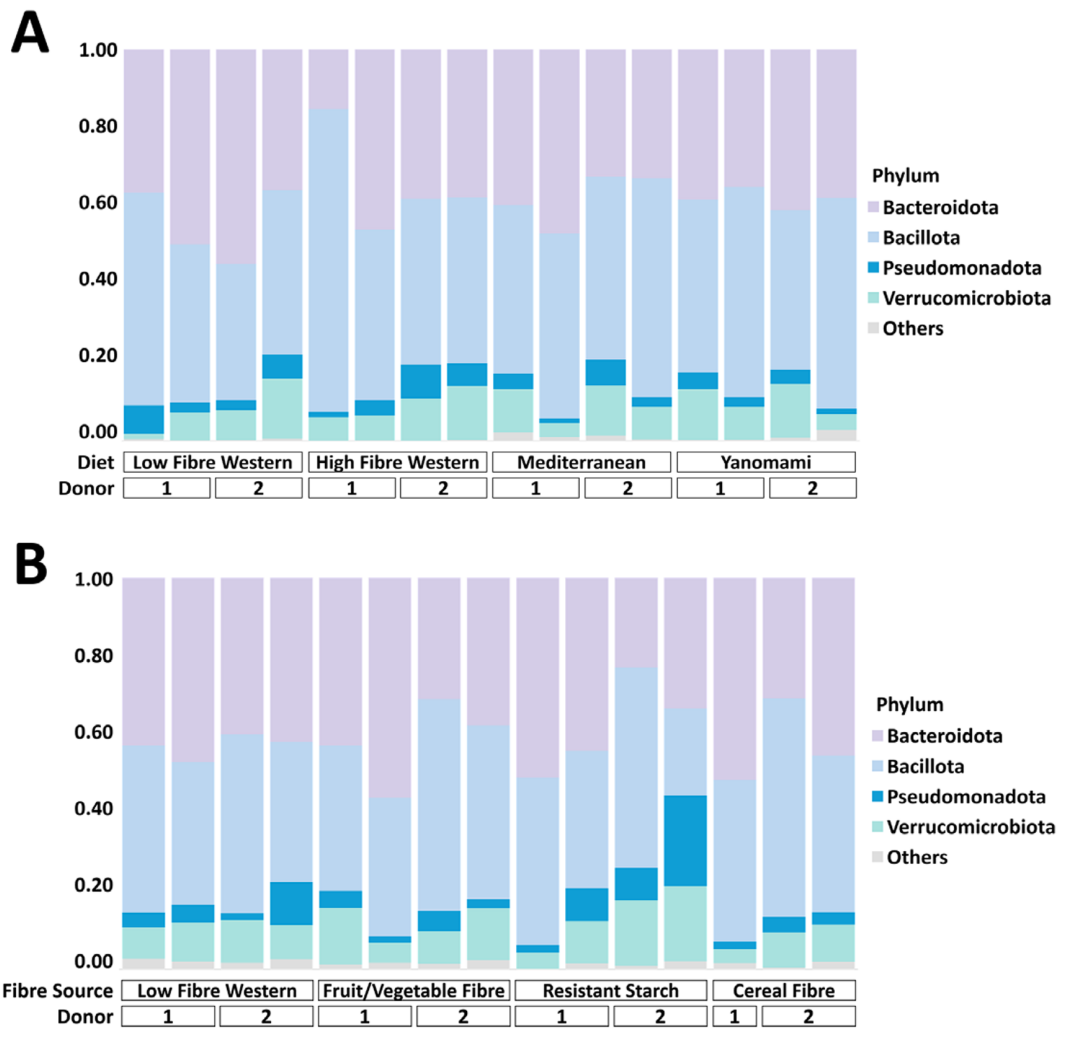


Figure 3. Changes in diets or fibre sources do not significantly alter the microbial composition of the gut microbiome. (A) Bioreactors were supplied with either the LFW, HFW, MED, or YAN diet media for 21 days. (B) Bioreactors were supplied with the FVF, RSF, or CRF diet media at Day 17 and grown for another 10 days; LFW had no added fibre. Effluents were collected at the end of each experiment, and an aliquot was subjected to 16S sequencing. The most dominant phyla found were Bacillota, Bacteroidota, and Verrucomicrobiota. Two biological replicates are shown for each donor, except for CRF for donor 1.

not perform statistical tests for each donor due to the limited number of biological replicates, we did observe some trends (Supplementary Figures S2 and S3). For the diet experiment, acetate and butyrate concentrations in both donors tended to be higher in HFW, MED, and YAN when compared to LFW. Propionate concentrations in both donors were highest in HFW but were similar in MED and YAN when compared to LFW (Supplementary Figure S2). For the fibre experiment, most SCFAs saw modest increases in concentrations following the addition of the fibres (Supplementary Figure S3). Overall, both donors had similar metabolite profiles and follow the general 60:20:20 ratio for the 3 major SCFAs. When both donors and replicates are pooled, it was revealed that acetate concentrations from the HFW ($p < 0.05$) and YAN ($p < 0.001$) diets, as well as the butyrate concentrations from the YAN diet ($p < 0.01$), were significantly different from the LFW diet (Figure 4C). Likewise, the acetate concentrations in the FVF fibre source were found to be much higher on Day 28 compared to Day 16 (Figure 4B) but were also

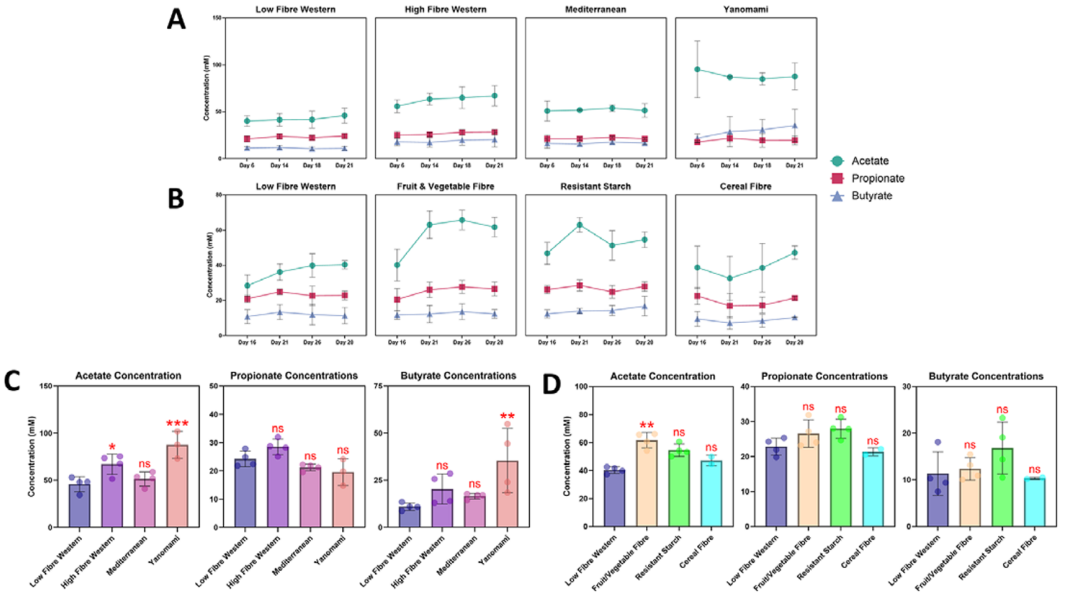


Figure 4. SCFA concentrations reach stability over time. Donors and biological replicates are pooled. (A-B) Metabolites were harvested at the indicated time points and submitted for 1-D ^{1}H NMR analysis. In the diet experiment, stability appears to be reached at Day 14, while in the fibre experiment where fibre was added at Day 17, stability appears to be reached at approximately Day 26. In both experiments, acetate concentrations were consistently the highest, followed by propionate then butyrate concentrations (except for YAN). Acetate concentrations were significantly higher on Day 28 when compared to Day 16 in the FVF fibre source (t (6) = 4.16, p = 0.025). (C-D) There was a significant effect of diet on acetate (F (3, 11) = 11.61, p = 0.001) and butyrate (F (3, 11) = 10.35, p = 0.003) in the diet experiment and a post-hoc test showed that acetate concentrations in HFW (p = 0.03) and YAN (p = 0.0006) and butyrate concentrations in YAN (p = 0.0042) were significantly different from LFW. A significant effect of fibre was found on acetate (F (3, 10) = 11.31, p = 0.0001), with a post-hoc revealing a significant difference between FVF and LFW acetate concentrations (p = 0.004). Error bars represent standard deviation (SD). * p < 0.05, ** p < 0.01, *** p < 0.001 using a one-way ANOVA or Kruskal-Wallis test.

significantly different from LFW on Day 28 (Figure 4D; p < 0.01). Collectively, these results suggest that diets and fibre impact microbial metabolite output while the microbial composition remains relatively stable.

The source of metabolites influences gene expression in the developing zebrafish

To better understand the contributions of diet on the developing nervous system, we took advantage of the zebrafish model where we previously demonstrated that bacterially derived metabolites are both necessary and sufficient for proper nervous system development (Rea et al., 2022). In the current paradigm, we added cell-free metabolites generated from the bioreactors to GF zebrafish embryos at 30 to 50% epiboly and then pooled 5 zebrafish larvae at 3dpf for qRT-PCR to assess changes in the expression of key neural and inflammatory markers (Figure 5).

Despite the changes observed in the metabolic profiles, this did not translate into significant changes in the expression of the four genes we evaluated; however, we did observe unique expression patterns between donors. When assessing metabolites derived from different diets, the expression of both *il-6* and *tnfα* tended to be higher in zebrafish embryos treated with donor 1 metabolites when compared to CV zebrafish embryos, while *fosab* expression was lower in zebrafish embryos treated with donor 2 metabolites (Figure 5A). For metabolites derived from different fibre sources, we observed that the expression of the four genes evaluated remained relatively consistent between donors, but with a trend of reduced *il-6* and *tnfα* expression in the RSF and CRF fibre sources for both donors. Unlike the diet metabolites, the pattern of expression observed between donors in the fibre experiment was comparable (Figure 5B).

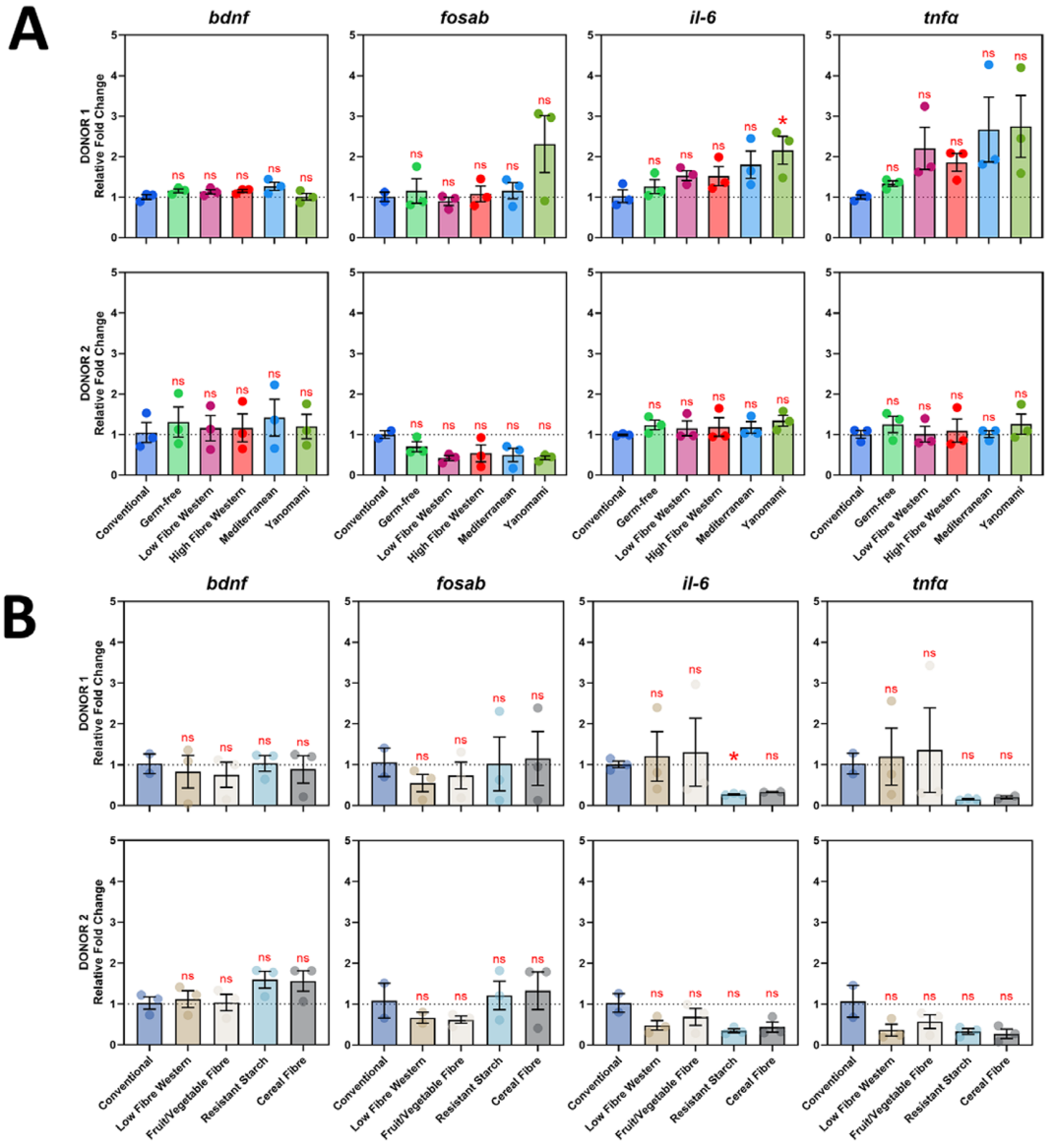


Figure 5. The effects of metabolites on the expression of neurodevelopmental and pro-inflammatory genes were limited, but distinct between donors. GF embryos were treated with metabolites at approximately 5hpf, harvested at 3dpf, and subjected to qRT-PCR. The expression of *bdnf*, *fosab*, *il-6*, and *tnfα* were measured, normalized to *ef1α*, and made relative to the CV group that had received no treatment. GF embryos that did not receive metabolite treatment were also evaluated. (A) Metabolites derived from diets did not seem to affect the expression of *bdnf* and *fosab*. Although no significant effect of metabolites treatment was observed, a post-hoc pairwise comparison revealed that the expression of *il-6* in YAN for donor 1 was significantly different compared to the CV group ($p = 0.032$). (B) Metabolites derived from different fibre source had minimal effects on the expression of the targeted genes, with the exception of *il-6* ($F(4, 9) = 9.70$, $p = 0.013$), noting a significant difference between CV and RSF groups ($p = 0.034$). $N = 3$. Error bars represent standard error of means (SEM). *$p < 0.05$ using a one-way ANOVA or Kruskal-Wallis test.

Taken together, these results indicate that the metabolites used in these experiments have limited impact on gene expression in the developing zebrafish nervous and immune systems, at least for the four genes we evaluated.

Metabolites can ameliorate some of the effects of stress

Given the neuroactive properties of diets high in fibre, we wanted to determine if the different diets can ameliorate the effects of stressors. We hypothesised that diets rich in fibre (HFW, MED, and YAN) would be able to assuage the effects of stress when compared to the diets LFW. To test this, we chose 3 stressors commonly used in zebrafish studies: swirling, osmotic, and heat (Alsop and Vijayan, 2008; Cheng et al., 2022; Lim and Bernier, 2022). First, we evaluated how the stressors affected gene expression in CV and GF zebrafish larvae at 3dpf. For the CV group, the swirling stress caused a significant increase in the expression of *bdnf* (Figure 6A, Controls; $p < 0.01$) and *tnfα* (Supplementary Figure S4A; $p < 0.05$). The osmotic and heat stress significantly increased the expression of *tnfα* only (Supplementary Figure S4B and S4C; $p < 0.05$ and $p < 0.01$, respectively). We then predicted that the GF group, devoid of microbes and metabolites, would have exacerbated gene expression; however, we only observed significant increases in *bdnf* (Figure 6A, Controls; $p < 0.05$) and *fosab* expression (Supplementary Figure S5A; $p < 0.01$) for the swirling stress, while we saw significant increases in *bdnf* (Figure 6B, Controls; $p < 0.05$) and *fosab* expression (Supplementary Figure S5C; $p < 0.05$) for the osmotic and heat stress, respectively. There were no significant changes in *il-6* expression for CV or GF under any of the stressors (Supplementary Figure S6). These results indicate that the expression of *bdnf* seems to be the most sensitive to the three stressors, with the swirling stressor having a greater effect than the other two stressors.

Next, we added the different metabolites to GF embryos and evaluated gene expression under the same stressors. While we observed a significant effect of diets, contrary to our hypothesis, we did not observe any diet-specific effects and the LFW diet-affected gene expression similar to the other diets (Figure 6). More specifically, we observed that the *bdnf* expression was rescued (i.e., reduced to non-significant levels) by metabolites derived from donor 2, but not donor 1 in the swirling stress (Figure 6A). In contrast, metabolites from both donors were able to rescue *bdnf* expression from the effects of osmotic stress (Figure 6B). Curiously, the heat stress did not seem to impact *bdnf* expression (Figure 6C). After evaluating the expression of *fosab*, *il-6*, and *tnfα*, it was revealed that the *fosab* had variable and inconsistent results (Supplementary Figure S5), while both *il-6* (Supplementary Figure S6) and *tnfα*

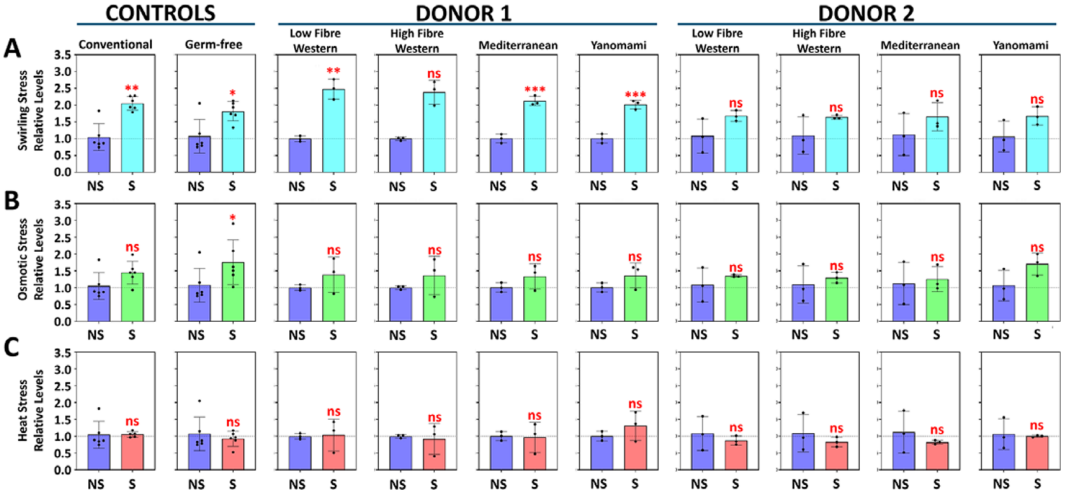


Figure 6. The metabolites can ameliorate the effects of stressors on *bdnf* expression. (A) A significant increase in *bdnf* expression was observed following an hour-long swirling stressor in the CV and GF groups. Similar findings were seen in the LFW, MED, and YAN groups for donor 1, but these were reduced to non-significant levels for donor 2. (B) Only the GF group saw a significant increase in *bdnf* expression following the osmotic stressor. The CV and all metabolite-treated groups were non-significant. (C) The heat stressor did not affect the expression of *bdnf* in all groups. $N = 6$ for controls, $N = 3$ for metabolite treatment groups. Error bars represent standard deviation * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ using an unpaired *t*-test or Mann–Whitney test.

(Supplementary Figure S4) seemed to be relatively stable following all three stressors. Overall, no discernible differences between diets could be identified. Nonetheless, we saw that *bdnf* expression appears to be amenable to both stressor and metabolites and, likewise, follows a donor-specific pattern of expression consistent with our previous results.

Discussion

Gut-derived microbial metabolites have gained increasing attention for their ability to influence the brain and behaviour (Cryan and Dinan, 2012; Mayer et al., 2015; Agustí et al., 2018; Appleton, 2018; Van de Wouw et al., 2018; Cryan et al., 2019; Marx et al., 2021; Horn et al., 2022; Loh et al., 2024; Ross et al., 2024; Schneider et al., 2024; Cryan, 2025; O'Riordan et al., 2025; Rosell-Cardona et al., 2025) but empirical evidence is lacking. Previous work in our lab demonstrated that metabolites are both necessary and sufficient for timely neurodevelopment. We found that, in the absence of microbes and their metabolites (i.e, germ-free), broad neural gene expression was delayed but was partially rescued by the addition of gut-derived zebrafish metabolites (Rea et al., 2022). Predicated on this work, here we used the GF zebrafish model to evaluate diet-derived metabolites on nervous system development.

Following the expansion of our selected donor faecal samples, we observed a decrease in Actinomycetota and an increase in Bacteroidota phyla. Although these phyla are common in typical gut microbiomes (Dethlefsen et al., 2008; Turnbaugh et al., 2009; Ubeda et al., 2010; Walker et al., 2011; Wu et al., 2011; David et al., 2014; David et al., 2014; Sonnenburg and Bäckhed, 2016; Wipperman et al., 2017; De Gunzburg et al., 2018; Palleja et al., 2018; Valdes et al., 2018; Cabral et al., 2020; Anthony et al., 2022), this shift may have made the microbial community less responsive to changes in diet or fibre supplementation (De Filippo et al., 2010; Ou et al., 2013; Wu et al., 2016; Biesiekierski et al., 2019; Leeming et al., 2019; Ard et al., 2020; Ostojic, 2020). Alternatively, the gut microbiome of healthy individuals is typically characterised by its resilience to perturbations (Dethlefsen et al., 2008; Bäckhed et al., 2012; Relman, 2012; Manrique et al., 2017), suggesting that the use of healthy donor samples may explain the lack of significant changes we observed. Because our study was limited to only two healthy donors, future iterations of this experiment should expand the number of donor faecal samples to increase both the sample size and statistical power, as well as include samples from diseased or unhealthy donors. In support of this, preliminary data from our lab has shown that GF zebrafish embryos treated with metabolites from an autistic donor have markedly different effects on gene expression when compared to non-autistic controls.

Despite the lack of microbial distinctions, we found several differences in the metabolic profiles between the diets and fibre sources in a pooled analysis. Specifically, differences in SCFA levels between LFW and the other diets and fibre sources were found, indicating that high-fibre diets or the supplementation of fibre can alter the metabolic profiles of the gut microbiome. Combined with the sequencing results, the differences in SCFA concentrations are strikingly similar to the observations reported by Wu et al. (2016). In their study, they report that healthy vegans and omnivores had similar gut microbial profiles. While they did not see significant differences in faecal SCFA concentrations after 10 days of dietary intervention, they attributed this to a high intersubject variability of baseline SCFA concentrations, suggesting that SCFA concentrations depend on the subject, consistent with the donor effects observed in our study. Further, work by Fragiadakis et al. (2020) revealed that dietary interventions caused substantial changes in participant gut microbiomes, but their microbiomes eventually returned near its baseline status despite maintaining the dietary interventions. Together, these studies corroborate our results, underscoring the resilience of the gut microbiome.

The gross similarities we observed in the microbial profiles between donors 1 and 2, along with the differences we saw in their metabolic profiles, suggest that subtleties in the microbiome allow individuals to respond differently to the diets and fibres. Given the neuroactive properties of metabolites (Cryan and Dinan, 2012; Mayer et al., 2015; Agustí et al., 2018; Appleton, 2018; Van de Wouw et al., 2018; Cryan et al., 2019; Marx et al., 2021; Horn et al., 2022; Loh et al., 2024; Ross et al., 2024; Schneider et al., 2024;

Cryan, 2025; O'Riordan et al., 2025; Rosell-Cardona et al., 2025), we hypothesised that diets high in fibre would positively affect the nervous system compared to diets low in fibre. To test this, we evaluated the expression of brain-derived neurotrophic factor (*bdnf*) and *fosab* (a zebrafish homolog of mammalian *fos*), which are excellent markers for neurodevelopment (Scharfman et al., 2005; Rossi et al., 2006; Appleyard, 2009; Bath et al., 2012; Bathina and Das, 2015; Colucci-D'Amato et al., 2020; Agnihotri and Mohajeri, 2022; Aparicio et al., 2022; Pisani et al., 2023; Delgado-Ocaña and Cuesta, 2024), as well as interleukin-6 (*il-6*) and tumour necrosis factor alpha (*tnfa*) which are reliable markers for stress and immune function (Sullivan et al., 2017; Liao et al., 2021; Miao et al., 2021; Franza et al., 2024). Overall, our results demonstrated that the metabolites have limited effects on the expression of the four genes evaluated; however, we observed donor-specific effects, particularly in the expression of *il-6* and *tnfa*. These results suggest that the effects of the metabolites are less dependent on the diet or fibre source but more influenced by its source microbiome (i.e., the donors).

Next, we subjected a pool of zebrafish larvae to one of three stressors and found a significant increase in *bdnf* expression in CV and GF larvae following an osmotic or swirling stress. When treated with metabolites, we found that all four diets were successful in rescuing *bdnf* expression in both donors following an osmotic stressor, but this rescue was only seen in larvae treated with donor 2 metabolites following a swirling stressor. Notably, the same patterns are not seen in the other genes evaluated. A study by Pate et al. (2023) found reductions of baseline *fos* in the prefrontal cortex of GF animals, whereas Yu et al. (2025) saw an increase in *fos* expression in the amygdala and hypothalamus of GF mice. Therefore, the variability of *fosab* expression in our experiments may be due to the use of the whole embryo instead of just the brain or specific brain regions.

While our results did not support our hypothesis that diets high in fibre would ameliorate the effects of stress, it did demonstrate that metabolites, in general, can potentially limit the effects of stress and that its source plays a larger role in determining its effects. More detailed gene-expression analyses may reveal significant subtleties between the diets. Additionally, our results demonstrated that while the microbial component of the gut microbiome is resilient to changes in diet or fibre source, their metabolic output is not, arguing that it is more likely the collective genetic complement of the gut microbiome that matters and not the individual microbial species. Despite the lack of distinctions between the different diets or fibre sources, our study has demonstrated that gut-derived microbial metabolites can modulate gene expression in response to specific stressors and that these effects are more dependent on the source of the metabolites rather than the composition of the diet.

Supplementary material. The supplementary material for this article can be found at http://doi.org/10.1017/gmb.2026.10033.

Data availability statement. The data that support the findings of this study are available from the corresponding author upon reasonable request. The data analysed during this study are publicly available in the NCBI BIOProject accession number PRJNA1391537.

Acknowledgements. We would like to thank Hagan AquaLab staff, Matt Cornish and Mike Davies, for their assistance with our zebrafish colony. Thanks to members of the Van Raay lab and thanks to members of the Allen-Vercoe lab for their assistance with everything microbial. The authors acknowledge the families who participated in the Guelph Family Health Study (GFHS), Angela Annis and Madeline Nixon for their work in coordinating the GFHS, and Adam Sadowski and Amar Laila for their data analytical expertise.

Author contributions. Conceptualisation: T.J.V.R. and E.A.V; Methodology: J.D.R.C., T.B., S.J.V., E.A.V., and T.J.V.R.; Investigation: J.D.R.C., B.K., M.S.H., P.N.G.T., T.B., S.C., S.J.V., and T.J.V.R.; Formal analysis: J.D.R.C., S.C., and T.J.V.R.; Visualisation: J.D.R.C and T.J.V.R.; Supervision: J.D.R.C., S.J.V., and T.J.V.R.; Writing/editing: J.D.R.C. and T.J.V.R.; Funding acquisition: D.W.L.M., E.A.V., and T.J.V.R.

Funding. We are grateful for the generosity of the Weston Family Foundation for their support on this project.

Ethics and animal approval. The samples were collected from participants of the Guelph Family Health Study in accordance with the University of Guelph's research ethics board approval (REB# 17-07-003). The University of Guelph's Animal Care Committee has approved the care and use of the animals for this study (Animal User Protocol #4871).

References

Agnihotri N and **Mohajeri MH** (2022) Involvement of intestinal microbiota in adult neurogenesis and the expression of brain-derived neurotrophic factor. *International Journal of Molecular Sciences* **23**(24), 15934. https://doi.org/10.3390/ijms232415934.

Agustí A, **García-Pardo MP**, **López-Almela I**, **Campillo I**, **Maes M**, **Romaní-Pérez M** and **Sanz Y** (2018) Interplay between the gut-brain axis, obesity and cognitive function. *Frontiers in Neuroscience* **12**, 329519. https://doi.org/10.3389/fnins.2018.00155.

Aleman RS, **Moncada M** and **Aryana KJ** (2023) Leaky gut and the ingredients that help treat it: A review. *Molecules* **28**(2), 619. https://doi.org/10.3390/molecules28020619.

Alsop D and **Vijayan MM** (2008) Development of the corticosteroid stress axis and receptor expression in zebrafish. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **294**(3), R711–R719. https://doi.org/10.1152/ajpregu.00671.2007.

Anthony WE, **Wang B**, **Sukhum KV**, **D'Souza AW**, **Hink T**, **Cass C**, **Seiler S**, **Reske KA**, **Coon C**, **Dubberke ER**, **Burnham C-AD**, **Dantas G** and **Kwon JH** (2022) Acute and persistent effects of commonly used antibiotics on the gut microbiome and resistome in healthy adults. *Cell Reports* **39**(2), 110649. https://doi.org/10.1016/j.celrep.2022.110649.

Aparicio SYL, **Fierro ADJL**, **Abreu GEA**, **Cárdenas RT**, **Hernández LIG**, **Ávila GAC**, **Durán FR**, **Aguilar MEH**, **Denes JM**, **Chi-Castañeda LD**, and **Estudillo CAP** (2022) Current opinion on the use of c-Fos in neuroscience. *NeuroSci* **3**(4), 687–702. https://doi.org/10.3390/neurosci3040050.

Appleton J (2018) The gut-brain axis: Influence of microbiota on mood and mental health. *Integrative Medicine: A Clinician's Journal* **17**(4), 28.

Appleyard SM (2009) Lighting up neuronal pathways: The development of a novel transgenic rat that identifies Fos-activated neurons using a red fluorescent protein. *Endocrinology* **150**(12), 5199–5201. https://doi.org/10.1210/en.2009-1234.

Ard JD, **Lewis KH**, **Cohen SS**, **Rothberg AE**, **Coburn SL**, **Loper J**, **Matarese L**, **Pories WJ** and **Periman S** (2020) Differences in treatment response to a total diet replacement intervention versus a food-based intervention: A secondary analysis of the OPTIWIN trial. *Obesity Science & Practice* **6**(6), 605–614. https://doi.org/10.1002/osp4.444.

Arumugam M, **Raes J**, **Pelletier E**, **le Paslier D**, **Yamada T**, **Mende DR**, **Fernandes GR**, **Tap J**, **Bruls T**, **Batto JM**, **Bertalan M**, **Borruel N**, **Casellas F**, **Fernandez L**, **Gautier L**, **Hansen T**, **Hattori M**, **Hayashi T**, **Kleerebezem M**, **Kurokawa K**, **Leclerc M**, **Levenez F**, **Manichanh C**, **Nielsen HB**, **Nielsen T**, **Pons N**, **Poulain J**, **Qin J**, **Sicheritz-Ponten T**, **Tims S**, **Torrents D**, **Ugarte E**, **Zoetendal EG**, **Wang J**, **Guarner F**, **Pedersen O**, **de Vos WM**, **Brunak S**, **Doré J**, **MetaHIT Consortium**, **Antolín M**, **Artiguenave F**, **Blottiere HM**, **Almeida M**, **Brechot C**, **Cara C**, **Chervaux C**, **Cultrone A**, **Delorme C**, **Denariaz G**, **Dervyn R**, **Foerstner KU**, **Friss C**, **van de Guchte M**, **Guedon E**, **Haimet F**, **Huber W**, **van Hylckama-Vlieg J**, **Jamet A**, **Juste C**, **Kaci G**, **Knol J**, **Lakhdari O**, **Layec S**, **le Roux K**, **Maguin E**, **Mérieux A**, **Melo Minardi R**, **M'rini C**, **Muller J**, **Oozeer R**, **Parkhill J**, **Renault P**, **Rescigno M**, **Sanchez N**, **Sunagawa S**, **Torrejon A**, **Turner K**, **Vandemeulebrouck G**, **Varela E**, **Winogradsky Y**, **Zeller G**, **Weissenbach J**, **Ehrlich SD** and **Bork P** (2011) Enterotypes of the human gut microbiome. *Nature* **473**(7346), 174–180. https://doi.org/10.1038/nature09944.

Asnicar F, **Berry SE**, **Valdes AM**, **Nguyen LH**, **Piccinno G**, **Drew DA**, **Leeming E**, **Gibson R**, **le Roy C**, **Khatib HA**, **Francis L**, **Mazidi M**, **Mompeo O**, **Valles-Colomer M**, **Tett A**, **Beghini F**, **Dubois L**, **Bazzani D**, **Thomas AM**, **Mirzayi C**, **Khleborodova A**, **Oh S**, **Hine R**, **Bonnett C**, **Capdevila J**, **Danzanvilliers S**, **Giordano F**, **Geistlinger L**, **Waldron L**, **Davies R**, **Hadjigeorgiou G**, **Wolf J**, **Ordovás JM**, **Gardner C**, **Franks PW**, **Chan AT**, **Huttenhower C**, **Spector TD** and **Segata N** (2021) Microbiome connections with host metabolism and habitual diet from 1,098 deeply phenotyped individuals. *Nature Medicine* **27**(2), 321–332. https://doi.org/10.1038/s41591-020-01183-8.

Babraham Bioinformatics (2010) FastQC: A quality control tool for high throughput sequence data. Available at http://www.bioinformatics.babraham.ac.uk/projects/fastqc. (Accessed 12 August 2022).

Bäckhed F, **Fraser CM**, **Ringel Y**, **Sanders ME**, **Sartor RB**, **Sherman PM**, **Versalovic J**, **Young V** and **Finlay BB** (2012) Defining a healthy human gut microbiome: Current concepts, future directions, and clinical applications. *Cell Host & Microbe* **12**(5), 611–622. https://doi.org/10.1016/j.chom.2012.10.012.

Barko PC, **McMichael MA**, **Swanson KS** and **Williams DA** (2018) The gastrointestinal microbiome: A review. *Journal of Veterinary Internal Medicine* **32**(1), 9–25. https://doi.org/10.1111/jvim.14875.

Bath KG, **Akins MR** and **Lee FS** (2012) BDNF control of adult SVZ neurogenesis. *Developmental Psychobiology* **54**(6), 578–589. https://doi.org/10.1002/dev.20546.

Bathina S and **Das UN** (2015) Brain-derived neurotrophic factor and its clinical implications. *Archives of Medical Science* **11**(6), 1164–1178. https://doi.org/10.5114/aoms.2015.56342.

Beghini F, **McIver LJ**, **Blanco-Míguez A**, **Dubois L**, **Asnicar F**, **Maharjan S**, **Mailyan A**, **Manghi P**, **Scholz M**, **Thomas AM**, **Valles-Colomer M**, **Weingart G**, **Zhang Y**, **Zolfo M**, **Huttenhower C**, **Franzosa EA** and **Segata N** (2021) Integrating taxonomic, functional, and strain-level profiling of diverse microbial communities with bioBakery 3. *eLife* **10**, e65088. https://doi.org/10.7554/eLife.65088.

Berding K, **Vlckova K**, **Marx W**, **Schellekens H**, **Stanton C**, **Clarke G**, **Jacka F**, **Dinan TG** and **Cryan JF** (2021) Diet and the microbiota–gut–brain axis: Sowing the seeds of good mental health. *Advances in Nutrition* **12**(4), 1239–1285. https://doi.org/10.1093/advances/nmaa181.

Biagi E, **Nylund L**, **Candela M**, **Ostan R**, **Bucci L**, **Pini E**, **Nikkïla J**, **Monti D**, **Satokari R**, **Franceschi C**, **Brigidi P** and **de Vos W** (2010) Through ageing, and beyond: Gut microbiota and inflammatory status in seniors and centenarians. *PLoS One* **5**(5), e10667. https://doi.org/10.1371/journal.pone.0010667.

Biesiekierski JR, **Jalanka J** and **Staudacher HM** (2019) Can gut microbiota composition predict response to dietary treatments? *Nutrients* **11**(5), 1134. https://doi.org/10.3390/nu11051134.

Bolger AM, **Lohse M** and **Usadel B** (2014) Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* **30**(15), 2114–2120. https://doi.org/10.1093/bioinformatics/btu170.

Cabral DJ, **Wurster JI**, **Korry BJ**, **Penumutchu S** and **Belenky P** (2020) Consumption of a Western-style diet modulates the response of the murine gut microbiome to ciprofloxacin. *Msystems* **5**(4), 10–1128. https://doi.org/10.1128/mSystems.00317-20.

Cani PD, **Neyrinck AM**, **Fava F**, **Knauf C**, **Burcelin RG**, **Tuohy KM**, **Gibson GR** and **Delzenne NM** (2007) Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. *Diabetologia* **50**(11), 2374–2383. https://doi.org/10.1007/s00125-007-0791-0.

Chen Y, **Zhou J** and **Wang L** (2021) Role and mechanism of gut microbiota in human disease. *Frontiers in Cellular and Infection Microbiology* **11**, 625913. https://doi.org/10.3389/fcimb.2021.625913.

Cheng R-K, **Tan JXM**, **Chua KX**, **Tan CJX** and **Wee CL** (2022) Osmotic stress uncovers correlations and dissociations between larval zebrafish anxiety endophenotypes. *Frontiers in Molecular Neuroscience* **15**, 900223. https://doi.org/10.3389/fnmol.2022.900223.

Cho I and **Blaser MJ** (2012) The human microbiome: At the interface of health and disease. *Nature Reviews Genetics* **13**(4), 260–270. https://doi.org/10.1038/nrg3182.

Collins S, **Dash S**, **Allender S**, **Jacka F** and **Hoare E** (2022) Diet and mental health during emerging adulthood: A systematic review. *Emerging Adulthood* **10**(3), 645–659. https://doi.org/10.1177/2167696820943028.

Colucci-D'Amato L, **Speranza L** and **Volpicelli F** (2020) Neurotrophic factor BDNF, physiological functions and therapeutic potential in depression, neurodegeneration and brain cancer. *International Journal of Molecular Sciences* **21**(20), 7777. https://doi.org/10.3390/ijms21207777.

Conteville LC, **Oliveira-Ferreira J** and **Vicente ACP** (2019) Gut microbiome biomarkers and functional diversity within an Amazonian semi-nomadic hunter–gatherer group. *Frontiers in Microbiology* **10**, 1743. https://doi.org/10.3389/fmicb.2019.01743.

Cronin P, **Joyce SA**, **O'Toole PW** and **O'Connor EM** (2021) Dietary fibre modulates the gut microbiota. *Nutrients* **13**(5), 1655. https://doi.org/10.3390/nu13051655.

Cryan JF (2025) Microbiome and brain development: A tale of two systems. *Annals of Nutrition and Metabolism* **81**(Suppl. 1), 34–46. https://doi.org/10.1159/000544950.

Cryan JF and **Dinan TG** (2012) Mind-altering microorganisms: The impact of the gut microbiota on brain and behaviour. *Nature Reviews Neuroscience* **13**(10), 701–712. https://doi.org/10.1038/nrn3346.

Cryan JF, **O'Riordan KJ**, **Cowan CSM**, **Sandhu KV**, **Bastiaanssen TFS**, **Boehme M**, **Codagnone MG**, **Cussotto S**, **Fulling C**, **Golubeva AV**, **Guzzetta KE**, **Jaggar M**, **Long-Smith CM**, **Lyte JM**, **Martin JA**, **Molinero-Perez A**, **Moloney G**, **Morelli E**, **Morillas E**, **O'Connor R**, **Cruz-Pereira JS**, **Peterson VL**, **Rea K**, **Ritz NL**, **Sherwin E**, **Spichak S**, **Teichman EM**, **van de Wouw M**, **Ventura-Silva AP**, **Wallace-Fitzsimons SE**, **Hyland N**, **Clarke G** and **Dinan TG** (2019) The microbiota-gut-brain axis. *Physiological Reviews* **99**(4), 1877–2013. https://doi.org/10.1152/physrev.00018.2018.

David LA, **Materna AC**, **Friedman J**, **Campos-Baptista MI**, **Blackburn MC**, **Perrotta A**, **Erdman SE** and **Alm EJ** (2014) Host lifestyle affects human microbiota on daily timescales. *Genome Biology* **15**(7), R89. https://doi.org/10.1186/gb-2014-15-7-r89.

David LA, **Maurice CF**, **Carmody RN**, **Gootenberg DB**, **Button JE**, **Wolfe BE**, **Ling AV**, **Devlin AS**, **Varma Y**, **Fischbach MA**, **Biddinger SB**, **Dutton RJ** and **Turnbaugh PJ** (2014) Diet rapidly and reproducibly alters the human gut microbiome. *Nature* **505**(7484), 559–563. https://doi.org/10.1038/nature12820.

Davis C, **Bryan J**, **Hodgson J** and **Murphy K** (2015) Definition of the Mediterranean diet: A literature review. *Nutrients* **7**(11), 9139–9153. https://doi.org/10.3390/nu7115459.

De Filippis DF, **Brigidi P**, **Cocolin L**, **Cagno DR**, **Ercolini D**, **Ferrocino I**, **Gobbetti M**, **Jeffery IB**, **La Storia A**, **Laghi L**, **Lazzi C**, **Neviani E**, **O'Toole PW**, **Pellegrini N**, **Serrazanetti DI**, **Turroni S** and **Vannini L** (2016) High-level adherence to a Mediterranean diet beneficially impacts the gut microbiota and associated metabolome. *Gut* **65**(11), 1812–1821. https://doi.org/10.1136/gutjnl-2015-309957.

De Filippo DC, **Cavalieri M**, **Collini S**, **Paola DM**, **Lionetti P**, **Massart S**, **Pieraccini G**, **Poullet JB** and **Ramazzotti M** (2010) Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proceedings of the National Academy of Sciences* **107**(33), 14691–14696. https://doi.org/10.1073/pnas.1005963107.

De Gunzburg J, **Alavoine L**, **Andremont A**, **Armand-Lefevre L**, **Augustin V**, **Burdet C**, **Chachaty E**, **Ducher A**, **Duval X**, **Ghozlane A**, **Kennedy S**, **Chatelier LE**, **Levenez F**, **Mentré F**, **Pons N**, **Ruppé E**, **Sablier-Gallis F**, **Varastet M** (2018) Protection of the human gut microbiome from antibiotics. *The Journal of Infectious Diseases* **217**(4), 628–636. https://doi.org/10.1093/infdis/jix604.

De Vos WM, **Cani PD**, **Tilg H** and **Hul VM** (2022) Gut microbiome and health: Mechanistic insights. *Gut* **71**(5), 1020–1032. https://doi.org/10.1136/gutjnl-2021-326789.

Deehan EC, **Duar RM**, **Armet AM**, **Perez-Muñoz ME**, **Jin M** and **Walter J** (2017) Modulation of the gastrointestinal microbiome with nondigestible fermentable carbohydrates to improve human health. *Microbiology Spectrum* **5**(5), 10–1128. https://doi.org/10.1128/microbiolspec.BAD-0019-2017.

Delgado-Ocaña S and **Cuesta S** (2024) From microbes to mind: Germ-free models in neuropsychiatric research. *MBio* **15**(10), e02075–e02024. https://doi.org/10.1128/mbio.02075-24.

Dethlefsen L, **Huse S**, **Sogin ML** and **Relman DA** (2008) The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. *PLoS Biology* **6**(11), e280. https://doi.org/10.1371/journal.pbio.0060280.

Duan Y, **Zeng L**, **Zheng C**, **Song B**, **Li F**, **Kong X** and **Xu K** (2018) Inflammatory links between high fat diets and diseases. *Frontiers in Immunology* **9**, 2649. https://doi.org/10.3389/fimmu.2018.02649.

Fragiadakis GK, **Wastyk HC**, **Robinson JL**, **Sonnenburg ED**, **Sonnenburg JL** and **Gardner CD** (2020) Long-term dietary intervention reveals resilience of the gut microbiota despite changes in diet and weight. *The American Journal of Clinical Nutrition* **111**(6), 1127–1136. https://doi.org/10.1093/ajcn/nqaa046.

Franza M, **Varricchio R**, **Alloisio G**, **de Simone G**, **di Bella S**, **Ascenzi P** and **di Masi A** (2024) Zebrafish (Danio rerio) as a model system to investigate the role of the innate immune response in human infectious diseases. *International Journal of Molecular Sciences* **25**(22), 12008. https://doi.org/10.3390/ijms252212008.

Gan Y, **Chen Y**, **Zhong H**, **Liu Z**, **Geng J**, **Wang H** and **Wang W** (2024) Gut microbes in central nervous system development and related disorders. *Frontiers in Immunology* **14**, 1288256. https://doi.org/10.3389/fimmu.2023.1288256.

Ganobis CM, **al-Abdul-Wahid MS**, **Renwick S**, **Yen S**, **Carriero C**, **Aucoin MG** and **Allen-Vercoe E** (2020) 1D ^{1}H NMR as a tool for fecal metabolomics. *Current Protocols in Chemical Biology* **12**(3), e83. https://doi.org/10.1002/cpch.83.

Gianetto-Hill CM, **Vancuren SJ**, **Daisley B**, **Renwick S**, **Wilde J**, **Schroeter K**, **Daigneault MC** and **Allen-Vercoe E** (2023) The Robogut: A bioreactor model of the human colon for evaluation of gut microbial community ecology and function. *Current Protocols* **3**(4), e737. https://doi.org/10.1002/cpz1.737.

Gill SK, **Rossi M**, **Bajka B** and **Whelan K** (2021) Dietary fibre in gastrointestinal health and disease. *Nature Reviews Gastroenterology & Hepatology* **18**(2), 101–116. https://doi.org/10.1038/s41575-020-00375-4.

Guan Z-W, **Yu E-Z** and **Feng Q** (2021) Soluble dietary fiber, one of the most important nutrients for the gut microbiota. *Molecules* **26**(22), 6802. https://doi.org/10.3390/molecules26226802.

Hamamah S and **Covasa M** (2022) Gut microbiota restores central neuropeptide deficits in germ-free mice. *International Journal of Molecular Sciences* **23**(19), 11756. https://doi.org/10.3390/ijms231911756.

Heslin AM and **McNulty B** (2023) Adolescent nutrition and health: Characteristics, risk factors and opportunities of an overlooked life stage. *Proceedings of the Nutrition Society* **82**(2), 142–156. https://doi.org/10.1017/S0029665123002689.

Horn J, **Mayer DE**, **Chen S** and **Mayer EA** (2022) Role of diet and its effects on the gut microbiome in the pathophysiology of mental disorders. *Translational Psychiatry* **12**(1), 164. https://doi.org/10.1038/s41398-022-01922-0.

Jennings A, **Kühn T**, **Bondonno NP**, **Waniek S**, **Bang C**, **Franke A**, **Kassubek J**, **Müller H-P**, **Both M**, **Weber KS**, **Lieb W** and **Cassidy A** (2024) The gut microbiome modulates associations between adherence to a Mediterranean-style diet, abdominal adiposity, and C-reactive protein in population-level analysis. *The American Journal of Clinical Nutrition* **119**(1), 136–144. https://doi.org/10.1016/j.ajcnut.2023.11.001.

Jørgensen BP, **Hansen AK**, **Kihl P**, **Nielsen DS**, **Sørensen DB**, **Wegener G** and **Winther G** (2015) Dietary magnesium deficiency affects gut microbiota and anxiety-like behaviour in C57BL/6N mice. *Acta Neuropsychiatrica* **27**(5), 307–311. https://doi.org/10.1017/neu.2015.10.

Kim CH (2023) Complex regulatory effects of gut microbial short-chain fatty acids on immune tolerance and autoimmunity. *Cellular & Molecular Immunology* **20**(4), 341–350. https://doi.org/10.1038/s41423-023-00987-1.

Koh A, **de Vadder F**, **Kovatcheva-Datchary P** and **Bäckhed F** (2016) From dietary fiber to host physiology: Short-chain fatty acids as key bacterial metabolites. *Cell* **165**(6), 1332–1345. https://doi.org/10.1016/j.cell.2016.05.041.

Langmead B and **Salzberg SL** (2012) Fast gapped-read alignment with Bowtie 2. *Nature Methods* **9**(4), 357–359. https://doi.org/10.1038/nmeth.1923.

Lassale C, **Batty GD**, **Baghdadli A**, **Jacka F**, **Sánchez-Villegas A**, **Kivimäki M** and **Akbaraly T** (2019) Healthy dietary indices and risk of depressive outcomes: A systematic review and meta-analysis of observational studies. *Molecular Psychiatry* **24**(7), 965–986. https://doi.org/10.1038/s41380-018-0237-8.

Lavelle A and **Sokol H** (2020) Gut microbiota-derived metabolites as key actors in inflammatory bowel disease. *Nature Reviews Gastroenterology & Hepatology* **17**(4), 223–237. https://doi.org/10.1038/s41575-019-0258-z.

Leeming ER, **Johnson AJ**, **Spector TD** and **le Roy CI** (2019) Effect of diet on the gut microbiota: Rethinking intervention duration. *Nutrients* **11**(12), 2862. https://doi.org/10.3390/nu11122862.

Ley RE, **Peterson DA** and **Gordon JI** (2006) Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell* **124**(4), 837–848. https://doi.org/10.1016/j.cell.2006.02.017.

Li Y, **Lv MR**, **Wei YJ**, **Sun L**, **Zhang JX**, **Zhang HG** and **Li B** (2017) Dietary patterns and depression risk: A meta-analysis. *Psychiatry Research* **253**, 373–382. https://doi.org/10.1016/j.psychres.2017.04.020.

Liao Z, **Lin D**, **Jia J**, **Cai R**, **Yu Y** and **Li W** (2021) Innate immune response to fasting and refeeding in the zebrafish kidney. *Biomolecules* **11**(6), 825. https://doi.org/10.3390/biom11060825.

Lim MY-T and **Bernier NJ** (2022) Zebrafish parental progeny investment in response to cycling thermal stress and hypoxia: Deposition of heat shock proteins but not cortisol. *Journal of Experimental Biology* **225**(21), jeb244715. https://doi.org/10.1242/jeb.244715.

Liu J, **Tan Y**, **Cheng H**, **Zhang D**, **Feng W** and **Peng C** (2022) Functions of gut microbiota metabolites, current status and future perspectives. *Aging and Disease* **13**(4), 1106–1126. https://doi.org/10.14336/AD.2022.0104.

Loh JS, **Mak WQ**, **Tan LKS**, **Ng CX**, **Chan HH**, **Yeow SH**, **Foo JB**, **Ong YS**, **How CW** and **Khaw KY** (2024) Microbiota–gut–brain axis and its therapeutic applications in neurodegenerative diseases. *Signal Transduction and Targeted Therapy* **9**(1), 37. https://doi.org/10.1038/s41392-024-01743-1.

Luczynski P, **McVey Neufeld K-A**, **Oriach CS**, **Clarke G**, **Dinan TG** and **Cryan JF** (2016) Growing up in a bubble: Using germ-free animals to assess the influence of the gut microbiota on brain and behavior. *International Journal of Neuropsychopharmacology* **19**(8), pyw020. https://doi.org/10.1093/ijnp/pyw020.

Macfarlane GT, **Macfarlane S** and **Gibson GR** (1998) Validation of a three-stage compound continuous culture system for investigating the effect of retention time on the ecology and metabolism of bacteria in the human colon. *Microbial Ecology* **35**(2), 180–187. https://doi.org/10.1007/s002489900072.

Malesza IJ, **Malesza M**, **Walkowiak J**, **Mussin N**, **Walkowiak D**, **Aringazina R**, **Bartkowiak-Wieczorek J** and **Mądry E** (2021) High-fat, western-style diet, systemic inflammation, and gut microbiota: A narrative review. *Cells* **10**(11), 3164. https://doi.org/10.3390/cells10113164.

Manrique P, **Dills M** and **Young MJ** (2017) The human gut phage community and its implications for health and disease. *Viruses* **9**(6), 141. https://doi.org/10.3390/v9060141.

Marx W, **Lane M**, **Hockey M**, **Aslam H**, **Berk M**, **Walder K**, **Borsini A**, **Firth J**, **Pariante CM**, **Berding K**, **Cryan JF**, **Clarke G**, **Craig JM**, **Su K-P**, **Mischoulon D**, **Gomez-Pinilla F**, **Foster JA**, **Cani PD**, **Thuret S**, **Staudacher HM**, **Sánchez-Villegas A**, **Arshad H**, **Akbaraly T**, **O'Neil A**, **Segasby T** and **Jacka FN** (2021) Diet and depression: Exploring the biological mechanisms of action. *Molecular Psychiatry* **26**(1), 134–150. https://doi.org/10.1038/s41380-020-00925-x.

Mayer EA, **Tillisch K** and **Gupta A** (2015) Gut/brain axis and the microbiota. *The Journal of Clinical Investigation* **125**(3), 926–938. https://doi.org/10.1172/JCI76304.

McDonald JAK, **Schroeter K**, **Fuentes S**, **Heikamp-deJong I**, **Khursigara CM**, **de Vos WM** and **Allen-Vercoe E** (2013) Evaluation of microbial community reproducibility, stability and composition in a human distal gut chemostat model. *Journal of Microbiological Methods* **95**(2), 167–174. https://doi.org/10.1016/j.mimet.2013.08.008.

McFall-Ngai M, **Hadfield MG**, **Bosch TCG**, **Carey HV**, **Domazet-Lošo T**, **Douglas AE**, **Dubilier N**, **Eberl G**, **Fukami T**, **Gilbert SF**, **Hentschel U**, **King N**, **Kjelleberg S**, **Knoll AH**, **Kremer N**, **Mazmanian SK**, **Metcalf JL**, **Nealson K**, **Pierce NE**, **Rawls JF**, **Reid A**, **Ruby EG**, **Rumpho M**, **Sanders JG**, **Tautz D** and **Wernegreen JJ** (2013) Animals in a bacterial world, a new imperative for the life sciences. *Proceedings of the National Academy of Sciences* **110**(9), 3229–3236. https://doi.org/10.1073/pnas.1218525110.

Miao KZ, **Kim GY**, **Meara GK**, **Qin X** and **Feng H** (2021) Tipping the scales with zebrafish to understand adaptive tumor immunity. *Frontiers in Cell and Developmental Biology* **9**, 660969. https://doi.org/10.3389/fcell.2021.660969.

Moles L and **Otaegui D** (2020) The impact of diet on microbiota evolution and human health. Is diet an adequate tool for microbiota modulation? *Nutrients* **12**(6), 1654. https://doi.org/10.3390/nu12061654.

Mueller NT, **Noya-Alarcon O**, **Contreras M**, **Appel LJ** and **Dominguez-Bello MG** (2018) Association of age with blood pressure across the lifespan in isolated Yanomami and Yekwana villages. *JAMA Cardiology* **3**(12), 1247–1249. https://doi.org/10.1001/jamacardio.2018.3676.

O'Riordan KJ, **Collins MK**, **Moloney GM**, **Knox EG**, **Aburto MR**, **Fülling C**, **Morley SJ**, **Clarke G**, **Schellekens H** and **Cryan JF** (2022) Short chain fatty acids: Microbial metabolites for gut-brain axis signalling. *Molecular and Cellular Endocrinology* **546**, 111572. https://doi.org/10.1016/j.mce.2022.111572.

O'Riordan KJ, **Moloney GM**, **Keane L**, **Clarke G** and **Cryan JF** (2025) The gut microbiota-immune-brain axis: Therapeutic implications. *Cell Reports Medicine* **6**(3), 101982. https://doi.org/10.1016/j.xcrm.2025.101982.

Ohland CL, **Kish L**, **Bell H**, **Thiesen A**, **Hotte N**, **Pankiv E** and **Madsen KL** (2013) Effects of Lactobacillus helveticus on murine behavior are dependent on diet and genotype and correlate with alterations in the gut microbiome. *Psychoneuroendocrinology* **38**(9), 1738–1747. https://doi.org/10.1016/j.psyneuen.2013.02.008.

Orlich MJ, **Singh PN**, **Sabaté J**, **Jaceldo-Siegl K**, **Fan J**, **Knutsen S**, **Beeson WL** and **Fraser GE** (2013) Vegetarian dietary patterns and mortality in Adventist Health Study 2. *JAMA Internal Medicine* **173**(13), 1230–1238. https://doi.org/10.1001/jamainternmed.2013.6473.

Ostojic SM (2020) Short-term GAA loading: Responders versus nonresponders analysis. *Food Science & Nutrition* **8**(8), 4446–4448. https://doi.org/10.1002/fsn3.1744.

Ou J, **Carbonero F**, **Zoetendal EG**, **DeLany JP**, **Wang M**, **Newton K**, **Gaskins HR** and **O'Keefe SJD** (2013) Diet, microbiota, and microbial metabolites in colon cancer risk in rural Africans and African Americans. *The American Journal of Clinical Nutrition* **98**(1), 111–120. https://doi.org/10.3945/ajcn.112.056689.

Palleja A, **Mikkelsen KH**, **Forslund SK**, **Kashani A**, **Allin KH**, **Nielsen T**, **Hansen TH**, **Liang S**, **Feng Q**, **Zhang C**, **Pyl PT**, **Coelho LP**, **Yang H**, **Wang J**, **Typas A**, **Nielsen MF**, **Nielsen HB**, **Bork P**, **Wang J**, **Vilsbøll T**, **Hansen T**, **Knop FK**,

Arumugam M and **Pedersen O** (2018) Recovery of gut microbiota of healthy adults following antibiotic exposure. *Nature Microbiology* **3**(11), 1255–1265. https://doi.org/10.1038/s41564-018-0257-9.

Pate T, **Anthony DC** and **Radford-Smith DE** (2023) cFOS expression in the prefrontal cortex correlates with altered cerebral metabolism in developing germ-free mice. *Frontiers in Molecular Neuroscience* **16**, 1155620. https://doi.org/10.3389/fnmol.2023.1155620.

Patterson E, **Cryan JF**, **Fitzgerald GF**, **Ross RP**, **Dinan TG** and **Stanton C** (2014) Gut microbiota, the pharmabiotics they produce and host health. *Proceedings of the Nutrition Society* **73**(4), 477–489. https://doi.org/10.1017/S0029665114001426.

Pham LN, **Kanther M**, **Semova I** and **Rawls JF** (2008) Methods for generating and colonizing gnotobiotic zebrafish. *Nature Protocols* **3**(12), 1862–1875. https://doi.org/10.1038/nprot.2008.186.

Pisani A, **Paciello F**, **del Vecchio V**, **Malesci R**, **de Corso E**, **Cantone E** and **Fetoni AR** (2023) The role of BDNF as a biomarker in cognitive and sensory neurodegeneration. *Journal of Personalized Medicine* **13**(4), 652. https://doi.org/10.3390/jpm13040652.

Postler TS and **Ghosh S** (2017) Understanding the holobiont: How microbial metabolites affect human health and shape the immune system. *Cell Metabolism* **26**(1), 110–130. https://doi.org/10.1016/j.cmet.2017.05.008.

Rea V, **Bell I**, **Ball T** and **van Raay T** (2022) Gut-derived metabolites influence neurodevelopmental gene expression and Wnt signaling events in a germ-free zebrafish model. *Microbiome* **10**(1), 132. https://doi.org/10.1186/s40168-022-01302-2.

Redondo-Useros N, **Nova E**, **González-Zancada N**, **Díaz LE**, **Gómez-Martínez S** and **Marcos A** (2020) Microbiota and lifestyle: A special focus on diet. *Nutrients* **12**(6), 1776. https://doi.org/10.3390/nu12061776.

Relman DA (2012) The human microbiome: Ecosystem resilience and health. *Nutrition Reviews* **70**(Suppl 1), S2–S9. https://doi.org/10.1111/j.1753-4887.2012.00489.x.

Rinninella E, **Raoul P**, **Cintoni M**, **Franceschi F**, **Miggiano GAD**, **Gasbarrini A** and **Mele MC** (2019) What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. *Microorganisms* **7**(1), 14. https://doi.org/10.3390/microorganisms7010014.

Rosell-Cardona C, **Leigh S-J**, **Knox E**, **Tirelli E**, **Lyte JM**, **Goodson MS**, **Kelley-Loughnane N**, **Aburto MR**, **Cryan JF** and **Clarke G** (2025) Acute stress-induced alterations in short-chain fatty acids: Implications for the intestinal and blood brain barriers. *Brain, Behavior, & Immunity-Health* **46**, 100992. https://doi.org/10.1016/j.bbih.2025.100992.

Ross FC, **Mayer DE**, **Horn J**, **Cryan JF**, **del Rio D**, **Randolph E**, **Gill CIR**, **Gupta A**, **Ross RP**, **Stanton C** and **Mayer EA** (2024) Potential of dietary polyphenols for protection from age-related decline and neurodegeneration: A role for gut microbiota? *Nutritional Neuroscience* **27**(9), 1058–1076. https://doi.org/10.1080/1028415X.2023.2298098.

Rossi C, **Angelucci A**, **Costantin L**, **Braschi C**, **Mazzantini M**, **Babbini F**, **Fabbri ME**, **Tessarollo L**, **Maffei L**, **Berardi N** and **Caleo M** (2006) Brain-derived neurotrophic factor (BDNF) is required for the enhancement of hippocampal neurogenesis following environmental enrichment. *European Journal of Neuroscience* **24**(7), 1850–1856. https://doi.org/10.1111/j.1460-9568.2006.05059.x.

Rostgaard-Hansen AL, **Esberg A**, **Dicksved J**, **Hansen T**, **Pelve E**, **Brunius C**, **Halkjær J**, **Tjønneland A**, **Johansson I** and **Landberg R** (2024) Temporal gut microbiota variability and association with dietary patterns: From the one-year observational diet, cancer, and health-next generations MAX study. *The American Journal of Clinical Nutrition* **119**(4), 1015–1026. https://doi.org/10.1016/j.ajcnut.2024.01.027.

Sabit H, **Tombuloglu H**, **Rehman S**, **Almandil NB**, **Cevik E**, **Abdel-Ghany S**, **Rashwan S**, **Abasiyanik MF** and **Yee Waye MM** (2021) Gut microbiota metabolites in autistic children: An epigenetic perspective. *Heliyon* **7**(1), e06105. https://doi.org/10.1016/j.heliyon.2021.e06105.

Salazar J, **Angarita L**, **Morillo V**, **Navarro C**, **Martínez MS**, **Chacín M**, **Torres W**, **Rajotia A**, **Rojas M**, **Cano C**, **Añez R**, **Rojas J** and **Bermudez V** (2020) Microbiota and diabetes mellitus: Role of lipid mediators. *Nutrients* **12**(10), 3039. https://doi.org/10.3390/nu12103039.

San Mauro Martin I, **Blanco López M**, **Blumenfeld Olivares JA**, **Borregon Rivilla E**, **Echeverry López M**, **Elortegui Pascual P**, **García Bernat M**, **Garicano Vilar E**, **Quevedo Santos Y** and **Rincón Barrado M** (2018) Nutritional and environmental factors in attention-deficit hyperactivity disorder (ADHD): A cross-sectional study. *Nutritional Neuroscience* **21**(9), 641–647. https://doi.org/10.1080/1028415X.2017.1331952.

Scharfman H, **Goodman J**, **Macleod A**, **Phani S**, **Antonelli C** and **Croll S** (2005) Increased neurogenesis and the ectopic granule cells after intrahippocampal BDNF infusion in adult rats. *Experimental Neurology* **192**(2), 348–356. https://doi.org/10.1016/j.expneurol.2004.11.016.

Schneider E, **O'Riordan KJ**, **Clarke G** and **Cryan JF** (2024) Feeding gut microbes to nourish the brain: Unravelling the diet–microbiota–gut–brain axis. *Nature Metabolism* **6**(8), 1454–1478. https://doi.org/10.1038/s42255-024-01108-6.

Shi H, **Ge X**, **Ma X**, **Zheng M**, **Cui X**, **Pan W**, **Zheng P**, **Yang X**, **Zhang P**, **Hu M**, **Hu T**, **Tang R**, **Zheng K**, **Huang X-F** and **Yu Y** (2021) A fiber-deprived diet causes cognitive impairment and hippocampal microglia-mediated synaptic loss through the gut microbiota and metabolites. *Microbiome* **9**(1), 223. https://doi.org/10.1186/s40168-021-01172-0.

Silva YP, **Bernardi A** and **Frozza RL** (2020) The role of short-chain fatty acids from gut microbiota in gut-brain communication. *Frontiers in Endocrinology* **11**, 508738. https://doi.org/10.3389/fendo.2020.00025.

Sonnenburg JL and **Bäckhed F** (2016) Diet–microbiota interactions as moderators of human metabolism. *Nature* **535**(7610), 56–64. https://doi.org/10.1038/nature18846.

Staller K, **Song M**, **Grodstein F**, **Whitehead WE**, **Matthews CA**, **Kuo B** and **Chan AT** (2018) Increased long-term dietary fiber intake is associated with a decreased risk of fecal incontinence in older women. *Gastroenterology* **155**(3), 661–667. https://doi.org/10.1053/j.gastro.2018.05.021.

Statovci D, **Aguilera M**, **MacSharry J** and **Melgar S** (2017) The impact of western diet and nutrients on the microbiota and immune response at mucosal interfaces. *Frontiers in Immunology* **8**, 838. https://doi.org/10.3389/fimmu.2017.00838.

Sullivan C, **Gabor KA**, **Jurczyszak D**, **Kim CH**, **Matty MA**, **Millard PJ** and **Tobin DM** (2017) Infectious disease models in zebrafish. *Methods in Cell Biology* **138**, 106–136. https://doi.org/10.1016/bs.mcb.2016.10.005.

Topping DL and **Clifton PM** (2001) Short-chain fatty acids and human colonic function: Roles of resistant starch and nonstarch polysaccharides. *Physiological Reviews* **81**(3), 1031–1064. https://doi.org/10.1152/physrev.2001.81.3.1031.

Tremaroli V and **Bäckhed F** (2012) Functional interactions between the gut microbiota and host metabolism. *Nature* **489**(7415), 242–249. https://doi.org/10.1038/nature11552.

Turnbaugh PJ, **Vanessa RK**, **Jeremiah FJ**, **Federico RE**, **Rob K** and **Jeffrey GI** (2009) The effect of diet on the human gut microbiome: A metagenomic analysis in humanized gnotobiotic mice. *Science Translational Medicine* **1**(6) 6ra14. https://doi.org/10.1126/scitranslmed.3000322.

Ubeda C, **Taur Y**, **Jenq RR**, **Equinda MJ**, **Son T**, **Samstein M**, **Viale A**, **Socci ND**, **van den Brink MRM**, **Kamboj M** and **Pamer EG** (2010) Vancomycin-resistant enterococcus domination of intestinal microbiota is enabled by antibiotic treatment in mice and precedes bloodstream invasion in humans. *The Journal of Clinical Investigation* **120**(12), 4332–4341. https://doi.org/10.1172/JCI43918.

Valdes AM, **Walter J**, **Segal E** and **Spector TD** (2018) Role of the gut microbiota in nutrition and health. *BMJ* **361**, k2179. https://doi.org/10.1136/bmj.k2179.

Van de Wouw M, **Boehme M**, **Clarke G**, **Cryan JF**, **Dinan TG**, **Lyte JM**, **O'Sullivan O**, **Stanton C**, **Strain C** and **Wiley N** (2018) Short-chain fatty acids: Microbial metabolites that alleviate stress-induced brain–gut axis alterations. *The Journal of Physiology* **596**(20), 4923–4944. https://doi.org/10.1113/JP276431.

Velázquez KT, **Enos RT**, **Bader JE**, **Sougiannis AT**, **Carson MS**, **Chatzistamou I**, **Carson JA**, **Nagarkatti P**, **Nagarkatti M** and **Murphy EA** (2019) Prolonged high-fat-diet feeding promotes non-alcoholic fatty liver disease and alters gut microbiota in mice. *World Journal of Hepatology* **11**(8), 619–637. https://doi.org/10.4254/wjh.v11.i8.619.

Ventriglio A, **Sancassiani F**, **Contu MP**, **Latorre M**, **di Slavatore M**, **Fornaro M** and **Bhugra D** (2020) Mediterranean diet and its benefits on health and mental health: A literature review. *Clinical Practice and Epidemiology in Mental Health: CP & EMH* **16**(1), 156–164. https://doi.org/10.2174/1745017902016010156.

Vieira-Silva S, **Falony G**, **Darzi Y**, **Lima-Mendez G**, **Garcia Yunta R**, **Okuda S**, **Vandeputte D**, **Valles-Colomer M**, **Hildebrand F**, **Chaffron S** and **Raes J** (2016) Species–function relationships shape ecological properties of the human gut microbiome. *Nature Microbiology* **1**(8), 1–8. https://doi.org/10.1038/nmicrobiol.2016.88.

Wali JA, **Jarzebska N**, **Raubenheimer D**, **Simpson SJ**, **Rodionov RN** and **O'Sullivan JF** (2020) Cardio-metabolic effects of high-fat diets and their underlying mechanisms—A narrative review. *Nutrients* **12**(5), 1505. https://doi.org/10.3390/nu12051505.

Walker AW, **Ince J**, **Duncan SH**, **Webster LM**, **Holtrop G**, **Ze X**, **Brown D**, **Stares MD**, **Scott P**, **Bergerat A**, **Louis P**, **McIntosh F**, **Johnstone AM**, **Lobley GE**, **Parkhill J** and **Flint HJ** (2011) Dominant and diet-responsive groups of bacteria within the human colonic microbiota. *The ISME Journal* **5**(2), 220–230. https://doi.org/10.1038/ismej.2010.118.

Wang L, **Wang H**, **Zhang B**, **Popkin BM** and **du S** (2020) Elevated fat intake increases body weight and the risk of overweight and obesity among Chinese adults: 1991–2015 trends. *Nutrients* **12**(11), 3272. https://doi.org/10.3390/nu12113272.

Wen L and **Duffy A** (2017) Factors influencing the gut microbiota, inflammation, and type 2 diabetes. *The Journal of Nutrition* **147**(7), 1468S–1475S. https://doi.org/10.3945/jn.116.240754.

Wipperman MF, **Fitzgerald DW**, **Juste MAJ**, **Taur Y**, **Namasivayam S**, **Sher A**, **Bean JM**, **Bucci V** and **Glickman MS** (2017) Antibiotic treatment for Tuberculosis induces a profound dysbiosis of the microbiome that persists long after therapy is completed. *Scientific Reports* **7**(1), 10767. https://doi.org/10.1038/s41598-017-10346-6.

Wu GD, **Chen J**, **Hoffmann C**, **Bittinger K**, **Chen Y-Y**, **Keilbaugh SA**, **Bewtra M**, **Knights D**, **Walters WA**, **Knight R**, **Sinha R**, **Gilroy E**, **Gupta K**, **Baldassano R**, **Nessel L**, **Li H**, **Bushman FD** and **Lewis JD** (2011) Linking long-term dietary patterns with gut microbial enterotypes. *Science* **334**(6052), 105–108. https://doi.org/10.1126/science.1208344.

Wu GD, **Compher C**, **Chen EZ**, **Smith SA**, **Shah RD**, **Bittinger K**, **Chehoud C**, **Albenberg LG**, **Nessel L**, **Gilroy E**, **Star J**, **Weljie AM**, **Flint HJ**, **Metz DC**, **Bennett MJ**, **Li H**, **Bushman FD** and **Lewis JD** (2016) Comparative metabolomics in vegans and omnivores reveal constraints on diet-dependent gut microbiota metabolite production. *Gut* **65**(1), 63–72. https://doi.org/10.1136/gutjnl-2014-308209.

Xiao W, **Zhang Q**, **Yu L**, **Tian F**, **Chen W** and **Zhai Q** (2022) Effects of vegetarian diet-associated nutrients on gut microbiota and intestinal physiology. *Food Science and Human Wellness* **11**(2), 208–217. https://doi.org/10.1016/j.fshw.2021.11.002.

Yu W, **Xiao Y**, **Jayaraman A**, **Yen Y-C**, **Lee HU**, **Pettersson S** and **Je HS** (2025) Microbial metabolites tune amygdala neuronal hyperexcitability and anxiety-linked behaviors. *EMBO Molecular Medicine* **17**(2), 249–264. https://doi.org/10.1038/s44321-024-00179-y.

Zeng W, **Jin Q** and **Wang X** (2023) Reassessing the effects of dietary fat on cardiovascular disease in China: A review of the last three decades. *Nutrients* **15**(19), 4214. https://doi.org/10.3390/nu15194214.

Cite this article: Capistrano JDR, Ketheeswaranathan B, Horn MS, Tran PNG, Ball T, Chirmade S, Vancuren SJ, Ma DWL, Walton K, Allen-Vercoe E and Van Raay TJ 2026. Diet-derived microbial metabolites modulate stress-responsive gene expression in germ-free zebrafish. *Gut Microbiome,* **7**, e16, 1–22. https://doi.org/10.1017/gmb.2026.10033

THE NUTRITION SOCIETY
Advancing Nutritional Science

CAMBRIDGE UNIVERSITY PRESS