

University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

---

Faculty Publications in Food Science and  
Technology

Food Science and Technology Department

---

2020

## Fecal Short-Chain Fatty Acid Concentrations Increase in Newly Paired Male Marmosets (*Callithrix jacchus*)

Lifeng Zhu

Mallory J. Suhr Van Haute

Haley R. Hassenstab

Caroline Smith

Devin J. Rose

*See next page for additional authors*

Follow this and additional works at: <https://digitalcommons.unl.edu/foodsciefacpub>



Part of the [Food Science Commons](#)

---

This Article is brought to you for free and open access by the Food Science and Technology Department at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Faculty Publications in Food Science and Technology by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

---

**Authors**

Lifeng Zhu, Mallory J. Suhr Van Haute, Haley R. Hassenstab, Caroline Smith, Devin J. Rose, Aaryn C. Must, Andrew K. Benson, and Jeffrey A. French

---



# Fecal Short-Chain Fatty Acid Concentrations Increase in Newly Paired Male Marmosets (*Callithrix jacchus*)

 Lifeng Zhu,<sup>a,b\*</sup> Mallory J. Suhr Van Haute,<sup>b,d</sup> Haley R. Hassenstab,<sup>a</sup> Caroline Smith,<sup>b,d</sup>  Devin J. Rose,<sup>b,d</sup>  
 Aaryn C. Mustoe,<sup>a,b,c</sup> Andrew K. Benson,<sup>b,d</sup>  Jeffrey A. French<sup>a,b,c</sup>

<sup>a</sup>Department of Biology, University of Nebraska at Omaha, Omaha, Nebraska, USA

<sup>b</sup>Nebraska Food for Health Center, University of Nebraska-Lincoln, Lincoln, Nebraska, USA

<sup>c</sup>Department of Psychology, University of Nebraska at Omaha, Omaha, Nebraska, USA

<sup>d</sup>Department of Food Science and Technology, University of Nebraska-Lincoln, Lincoln, Nebraska, USA

**ABSTRACT** The role by which the gut microbiome influences host health (e.g., energy equilibrium and immune system) may be partly mediated by short-chain fatty acids, which are bacterial fermentation products from the dietary fibers. However, little is known about longitudinal changes in gut microbiome metabolites during cohabitation alongside social contact. In common marmosets (*Callithrix jacchus*), the gut microbiome community is influenced by social contact, as newly paired males and females develop convergent microbial profiles. Here, we monitored the dynamics of short-chain fatty acid concentrations in common marmoset feces from the preparing (PRE) to postpairing (POST) stages. In males, we observed that the concentrations of acetate, propionate, isobutyrate, and isovalerate significantly increased in the POST stage compared to the PRE stage. However, no significant changes were found in females. We further found that the propionate concentration was significantly positively correlated with the abundance of *Phascolarctobacterium* in the male feces. Thus, the sex difference in the changes in the concentrations of short-chain fatty acids might be related to sex-biased gut microbiome transmission after pairing. We suggest that the significant changes in the gut microbiomes and some short-chain fatty acids of the common marmoset during cohabitation may contribute to physiological homeostasis during pairing.

**IMPORTANCE** This study addressed a knowledge gap about longitudinal changes in the gut microbiome metabolites during animal pairing. This research in the laboratory common marmoset can control for the confounding factors such as diet and other environmental conditions. *Phascolarctobacterium* showed the highest contribution to the sex-biased transmission of the female to the male after pairing. Here, we observed the sex difference in the increase in short-chain fatty acid concentration in the feces of newly paired marmosets, which may be caused by the sex-biased gut microbiome transmission after pairing.

**KEYWORDS** common marmosets, cohabitation and social contact, short-chain fatty acids, sex difference

**M**armosets (*Callithrix jacchus*) are family-living primates, with groups consisting of adult males and females, who form long-term socially monogamous and cooperative breeding relationships, and their offspring. The principal bacteria in the marmoset gut microbiome include *Firmicutes* (~39%), *Bacteroidetes* (~29%), and *Actinobacteria* (~27%) (1). We have previously reported that newly paired marmosets displayed a significant convergence in their gut microbiomes during the first 8 weeks of cohabitation: significantly increase in the abundance of five genera (*Phascolarctobacterium*, *Alloprevotella*, *Anaerobiospirillum*, *Sutterella*, and *Coprobacter*) in both male

**Citation** Zhu L, Suhr Van Haute MJ, Hassenstab HR, Smith C, Rose DJ, Mustoe AC, Benson AK, French JA. 2020. Fecal short-chain fatty acid concentrations increase in newly paired male marmosets (*Callithrix jacchus*). *mSphere* 5: e00794-20. <https://doi.org/10.1128/mSphere.00794-20>.

**Editor** Vincent B. Young, University of Michigan-Ann Arbor

**Copyright** © 2020 Zhu et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Lifeng Zhu, zhulf2020@126.com.

\* Present address: Lifeng Zhu, College of Life Sciences, Nanjing Normal University, Nanjing, China.

**Received** 5 August 2020

**Accepted** 9 September 2020

**Published** 16 September 2020

and female feces after pairing (1). This finding may be associated with their social contact and affiliative behavior (e.g., social grooming, genital investigation, social approach, and mounts and copulations) (2–4). In addition, considering the pairing experiment performed at the same time, the unknown factors other than pairing could also have affected gut microbiome changes.

*Phascolarctobacterium* showed the highest contribution in the gut microbiome transmission in the newly paired marmosets, and this microbiome group exhibited the highest contribution to the sex-biased transmission pattern of the female to the male (1). Some studies have suggested that the transmitted gut microbiome within primate social groups may be beneficial to the host health, but with no direct evidence (5, 6).

The gut microbiome plays an important role in host immune development and health (7, 8). Short-chain fatty acids (SCFAs) are the end products of fermentation of dietary fibers and protein by the gut microbiome (9–12). SCFAs (including acetate [ACE], propionate [PA], butyrate [BUTY], isobutyrate [ISOB], and isovalerate [ISOV]) have a key role in the maintenance of intestinal homeostasis and help maintain energy equilibrium, immune system function, and health (9–12). For example, PA can increase host serotonin (5-hydroxytryptamine) biosynthesis in the intestine and affect gut motility and hemostasis (10). PA also reduces host inflammation, enhances tissue insulin sensitivity, maintains homeostasis of glucose, and induces hormone release (e.g., of leptin) (11, 12).

*Phascolarctobacterium* can produce short-chain fatty acids, such as propionate (PA) (13). Based on the significant elevation in *Phascolarctobacterium* following social pairing in marmosets, we hypothesized that these changes in gut microbiome community following pairing would result in significant increases in production of SCFAs, especially PA.

We measured the concentration of five SCFAs (acetate [ACE], propionate [PA], butyrate [BUTY], isobutyrate [ISOB], and isovalerate [ISOV]) from 228 fecal samples. The main SCFAs in these samples were ACE (mean:  $44.91 \pm 1.369 \mu\text{mol/g}$ ), PA (mean:  $10.86 \pm 0.331 \mu\text{mol/g}$ ), and BUTY (mean:  $2.75 \pm 0.151 \mu\text{mol/g}$ ). The concentrations of the other two SCFAs were very low (ISOB:  $0.35 \pm 0.298 \mu\text{mol/g}$ ; ISOC:  $0.41 \pm 0.341 \mu\text{mol/g}$ ). We compared the SCFA concentration from preparing (PRE) to postpairing (POST) for each sex. In males, we observed that the concentrations of ACE, PA, ISOB, and ISOV significantly increased in the POST stage compared to the PRE stage. No significant changes were found in female fecal samples after pairing, but the changes in PA after pairing were at the marginally significant level (Table 1, Benjamini-Hochberg [B-H] correction). Although there were significant increases in the abundance of five genera (*Phascolarctobacterium*, *Alloprevotella*, *Anaerobiospirillum*, *Sutterella*, and *Coprobacter*) in fecal samples from both males and females, the PA concentration was significantly associated only with the abundance of *Phascolarctobacterium* in fecal samples from male marmosets (Fig. 1, B-H correction). Therefore, we speculated that the highest contribution of *Phascolarctobacterium* to the sex-biased transmission pattern of the female to the male after pairing might result in the sex differences in the abundance changes of some SCFA concentrations.

*Phascolarctobacterium* bacteria are Gram-negative, obligately anaerobic, and non-spore-forming environmental microorganisms and have been found in soil, water, and some mammal feces (e.g., koala, human, and nonhuman primate) (1, 13–15). One of the main end products of the fermentation in *Phascolarctobacterium* is PA (13), which may have beneficial effects on the host (10–12, 16–18). For example, *Phascolarctobacterium* is positively correlated with the human positive mood (17), and *Phascolarctobacterium* has beneficial effects on host health and putatively decreases susceptibility to hepatic steatosis based on a rat model of nonalcoholic fatty liver (18). Moreover, PA is the essential mediator in the link between host nutrition, symbiotic gut microbiomes, and host physiology (19, 20). The purpose of physiological homeostasis is to neutralize or repair disturbance and to maintain stability (e.g., blood glucose level and blood pressure) (21). In addition, several diseases in humans (e.g., diabetes, obesity, and

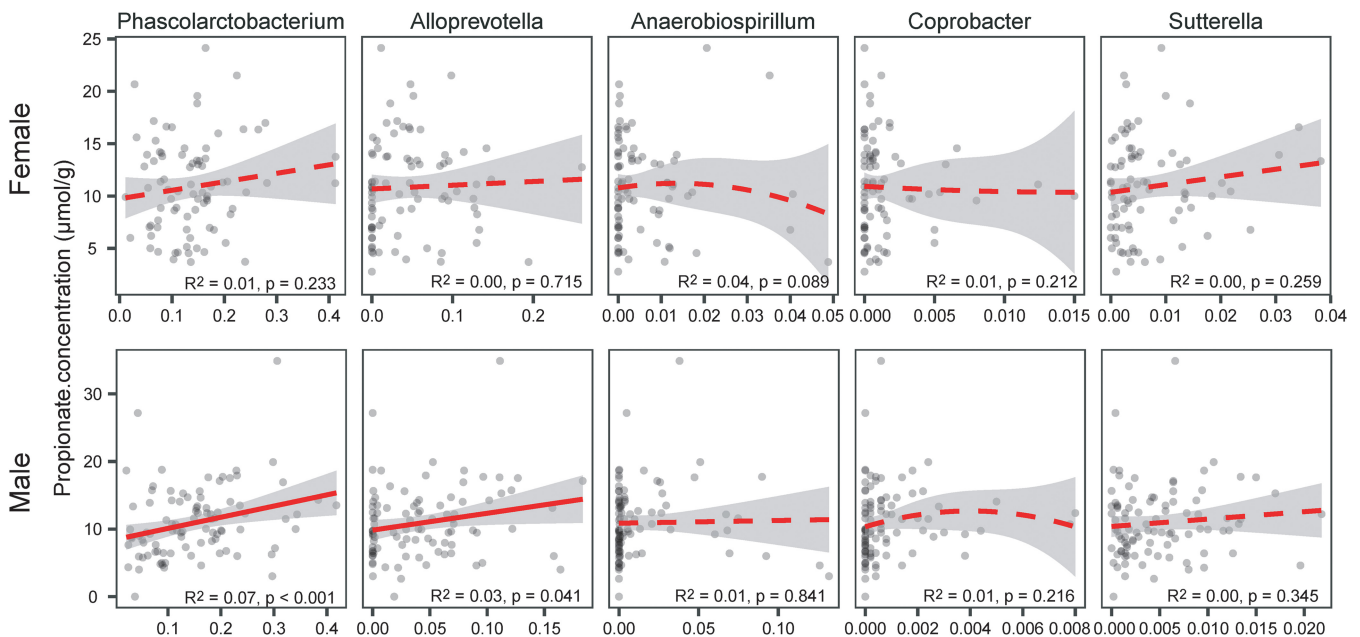
**TABLE 1** Average concentrations of the main short-chain fatty acids between PRE and POST stages<sup>a</sup>

Stage	Female						Male					
	Pair	Avg concn (μmol/g) of SCFA:					Pair	Avg concn (μmol/g) of SCFA:				
		ACE	PA	BUTY	ISOB	ISOV		ACE	PA	BUTY	ISOB	ISOV
PRE	P1	32.276	6.589	2.521	0.372	0.511	P1	43.541	8.295	2.583	0.405	0.574
	P2	33.776	11.238	1.110	0.229	0.301	P2	38.163	10.448	4.359	0.044	0.106
	P3	30.114	9.722	1.059	0.673	0.730	P3	41.156	14.046	3.994	0.547	0.596
	P4	32.658	8.962	0.618	0.062	0.019	P4	41.897	11.548	1.786	0.283	0.283
	P6	47.386	10.619	4.511	0.256	0.383	P6	30.623	6.957	0.849	0.099	0.123
	P7	39.489	9.689	2.639	0.273	0.323	P7	39.194	8.065	1.515	0.376	0.211
	P8	44.570	6.651	3.818	0.153	0.361	P8	35.155	8.191	0.810	0.126	0.098
	P9	40.672	7.107	2.040	0.000	0.043	P9	38.607	2.172	2.871	0.000	0.027
	POST	P1	35.469	12.332	1.768	0.607	0.644	P1	50.912	10.543	2.908	0.520
P2		43.088	13.829	2.903	0.248	0.288	P2	55.097	10.527	3.417	0.345	0.532
P3		20.327	8.337	0.928	0.158	0.195	P3	54.263	18.076	3.102	0.479	0.510
P4		41.029	11.197	2.116	0.605	0.657	P4	48.224	13.614	3.638	0.384	0.476
P6		73.356	15.450	7.412	0.400	0.459	P6	49.268	11.374	2.276	0.495	0.224
P7		50.789	12.073	3.576	0.546	0.660	P7	38.472	11.733	1.148	0.501	0.538
P8		59.063	12.432	5.889	0.142	0.370	P8	33.851	11.686	2.135	0.469	0.513
P9		47.334	12.822	2.552	0.517	0.616	P9	54.085	7.554	3.210	0.313	0.489
Wilcoxon paired test			0.078 <sup>NS</sup>	0.016 <sup>NS</sup>	0.055 <sup>NS</sup>	0.148 <sup>NS</sup>	0.195 <sup>NS</sup>		0.039*	0.008*	0.461 <sup>NS</sup>	0.016*

<sup>a</sup>The main short-chain fatty acids in this study include acetate (ACE), propionate (PA), butyrate (BUTY), isobutyrate (ISOB), and isovalerate (ISOV). Wilcoxon paired test, uncorrected *P* value based on Wilcoxon paired test. \*, significance after Benjamini-Hochberg correction ( $\alpha = 0.05$ ) within each sex. NS, nonsignificance after Benjamini-Hochberg correction ( $\alpha > 0.05$ ) within each sex.

autoimmune disorders) are associated with reduced SCFA production in the gut (22–25).

Interestingly, the establishment of social bonds within pairmates (e.g., pair bonding) is important for maintaining physiological homeostasis (26). The data presented here extend these findings to the sex difference in changes in the fermentation metabolites



**FIG 1** Concentrations of propionate (μmol/g, y axis) and the relative abundance of five genera (*Phascolarctobacterium*, *Alloprevotella*, *Anaerobiospirillum*, *Sutterella*, and *Coprobacter*). Dashed line, significant correlation based on the uncorrected *P* value. Solid line, nonsignificant correlation based on the uncorrected *P* value. The propionate concentration was significantly associated only with the abundance of *Phascolarctobacterium* in fecal samples from males after Benjamini-Hochberg correction ( $\alpha = 0.05$ ).

of the gut microbiome during pair formation. Although the fecal SCFAs do not entirely reflect the total intestinal production (19, 20), examining SCFAs in common marmoset feces is a possible way (noninvasive method) to show SCFA metabolism in the intestine indirectly. We suggest that the gut microbiome metabolites in marmosets may have effects on the host physiology after pairing. Therefore, this study provides the new insight that one of the putative mechanisms between social bonding and physiological homeostasis in humans and nonhuman primates might involve microbial transmission in the gut and the associated metabolites under the condition of sex difference.

**Newly paired marmoset experiment.** We examined fecal short-chain fatty acid concentrations in eight adult common marmoset pairs (eight females and eight males, aged 1.5 to 7.5 years). Baseline fecal samples (PRE; 59 fecal samples) were collected during a 2-week period prior to pairing (PRE), during which marmosets resided with an opposite-sex partner or in a family group (1). Marmosets were then rehoused in a new enclosure with a new opposite-sex partner who was a previously unfamiliar and unrelated marmoset. Fecal samples were collected in the postpairing phase (POST; 169 fecal samples) for an 8-week period following pairing. During both phases, marmosets were fed a consistent diet (commercial marmoset diet [Zupreem; Science Diet], *Tenebrio* larvae, scrambled eggs, fruits [red apple and cantaloupe], and gum arabic [Mazuri]). Fresh fecal samples were collected from marmosets in sterilized aluminum pans immediately after the light-on phase of the photoperiod. Samples were snap-frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$ . Most of these fecal samples were used in the previous gut microbiome study (1). This study was performed following the guidelines of the University of Nebraska Medical Center and the University of Nebraska at Omaha Institutional Animal Care and Use Committee. The protocol was approved by the University of Nebraska Medical Center/University of Nebraska at Omaha Institutional Animal Care and Use Committee (16-104).

**SCFA measurement.** Fecal samples were taken from the  $-80^{\circ}\text{C}$  freezer and thawed at room temperature. Fecal samples (0.21 g) were homogenized with 1 ml sterile phosphate-buffered saline and then centrifuged ( $10,000 \times g$ , 5 min). Next, 0.4 ml of supernatant from each sample was mixed with 0.1 ml of internal standard (7 mM 2-ethylbutyrate) followed by acidification, extraction into diethyl ether, and quantification by gas chromatography (Clarus 580; PerkinElmer, Waltham, MA, USA) based on the standard protocol (27). For each pair, we estimated two single average points, one from the PRE stage, the other from the POST stage (days after pairing: from 29 to 55). Wilcoxon paired tests were used to calculate the *P* value from these two groups (PRE versus POST) within each sex. The relationships between the abundance of the five gut microbiome genera (significantly increased in both females and males after pairing) and the concentration of PA were tested via linear and quadratic models. The better model was selected according to the lower value of Akaike's information criterion (28). False discovery rates were addressed by the Benjamini-Hochberg procedure (B-H correction,  $\alpha = 0.05$ ) for multiple-comparison testing within each sex (29). The above statistical analysis and plotting were conducted in R software (30).

## ACKNOWLEDGMENTS

We are grateful to members of the French lab for helping with sample collection and providing feedback during manuscript preparation. We are grateful to Qibin Gao and Wenqian Zhao for helping with the statistics analysis during manuscript revision.

The Callitrichid Research Center is supported in part by the NIH (HD089147).

L.Z., A.C.M., D.J.R., A.K.B., and J.A.F. designed the research. H.R.H. collected the fecal samples. L.Z., M.J.S.V.H., and C.S. carried out the experiment. L.Z. performed the data analysis. L.Z., A.C.M., and J.A.F. wrote the manuscript. Each author contributed to the discussion.

The authors declared no conflicts of interest relevant to this paper.

## REFERENCES

- Zhu L, Clayton JB, Suhr Van Haute MJ, Yang Q, Hassenstab HR, Mustoe AC, Knights D, Benson AK, French JA. 2020. Sex bias in gut microbiome transmission in newly paired marmosets (*Callithrix jacchus*). *mSystems* 5:e00910-19. <https://doi.org/10.1128/mSystems.00910-19>.
- Evans S, Poole T. 1983. Pair-bond formation and breeding success in the common marmoset *Callithrix jacchus jacchus*. *Int J Primatol* 4:83–97. <https://doi.org/10.1007/BF02739361>.
- Schaffner CM, Shepherd RE, Santos CV, French JA. 1995. Development of heterosexual relationships in wild black tufted-ear marmosets (*Callithrix kuhli*). *Am J Primatol* 36:185–200. <https://doi.org/10.1002/ajp.1350360303>.
- Evans S. 1983. The pair-bond of the common marmoset, *Callithrix jacchus jacchus*: an experimental investigation. *Anim Behav* 31:651–658. [https://doi.org/10.1016/S0003-3472\(83\)80220-6](https://doi.org/10.1016/S0003-3472(83)80220-6).
- Amato KR, Van Belle S, Di Fiore A, Estrada A, Stumpf R, White B, Nelson KE, Knight R, Leigh SR. 2017. Patterns in gut microbiota similarity associated with degree of sociality among sex classes of a neotropical primate. *Microb Ecol* 74:250–258. <https://doi.org/10.1007/s00248-017-0938-6>.
- Raulo A, Ruokolainen L, Lane A, Amato K, Knight R, Leigh S, Stumpf R, White B, Nelson KE, Baden AL, Tecot SR. 2018. Social behaviour and gut microbiota in red-bellied lemurs (*Eulemur rubriventer*): in search of the role of immunity in the evolution of sociality. *J Anim Ecol* 87:388–399. <https://doi.org/10.1111/1365-2656.12781>.
- Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, Magris M, Hidalgo G, Baldassano RN, Anokhin AP, Heath AC, Warner B, Reeder J, Kuczynski J, Caporaso JG, Lozupone CA, Lauber C, Clemente JC, Knights D, Knight R, Gordon JI. 2012. Human gut microbiome viewed across age and geography. *Nature* 486:222–227. <https://doi.org/10.1038/nature11053>.
- Gilbert JA, Blaser MJ, Caporaso JG, Jansson JK, Lynch SV, Knight R. 2018. Current understanding of the human microbiome. *Nat Med* 24:392–400. <https://doi.org/10.1038/nm.4517>.
- Corrêa-Oliveira R, Fachi JL, Vieira A, Sato FT, Vinolo MAR. 2016. Regulation of immune cell function by short-chain fatty acids. *Clin Transl Immunol* 5:e73. <https://doi.org/10.1038/cti.2016.17>.
- Yano JM, Yu K, Donaldson GP, Shastri GG, Ann P, Ma L, Nagler CR, Ismagilov RF, Mazmanian SK, Hsiao EY. 2015. Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell* 161:264–276. <https://doi.org/10.1016/j.cell.2015.02.047>.
- Xiong Y, Miyamoto N, Shibata K, Valasek MA, Motoike T, Kedziński RM, Yanagisawa M. 2004. Short-chain fatty acids stimulate leptin production in adipocytes through the G protein-coupled receptor GPR41. *Proc Natl Acad Sci U S A* 101:1045–1050. <https://doi.org/10.1073/pnas.2637002100>.
- Puddu A, Sanguineti R, Montecucco F, Viviani GL. 2014. Evidence for the gut microbiota short-chain fatty acids as key pathophysiological molecules improving diabetes. *Mediators Inflamm* 2014:162021. <https://doi.org/10.1155/2014/162021>.
- Watanabe Y, Nagai F, Morotomi M. 2012. Characterization of *Phascolarctobacterium succinatutens* sp. nov., an asaccharolytic, succinate-utilizing bacterium isolated from human feces. *Appl Environ Microbiol* 78:511–518. <https://doi.org/10.1128/AEM.06035-11>.
- Wu F, Guo X, Zhang J, Zhang M, Ou Z, Peng Y. 2017. *Phascolarctobacterium faecium* abundant colonization in human gastrointestinal tract. *Exp Ther Med* 14:3122–3126. <https://doi.org/10.3892/etm.2017.4878>.
- Rieke EL, Soupier ML, Moorman TB, Yang F, Howe A. 2018. Temporal dynamics of bacterial communities in soil and leachate water after swine manure application. *Front Microbiol* 9:3197. <https://doi.org/10.3389/fmicb.2018.03197>.
- Zhang X, Zhao Y, Xu J, Xue Z, Zhang M, Pang X, Zhang X, Zhao L. 2015. Modulation of gut microbiota by berberine and metformin during the treatment of high-fat diet-induced obesity in rats. *Sci Rep* 5:14405. <https://doi.org/10.1038/srep14405>.
- Li L, Su Q, Xie B, Duan L, Zhao W, Hu D, Wu R, Liu H. 2016. Gut microbes in correlation with mood: case study in a closed experimental human life support system. *Neurogastroenterol Motil* 28:1233–1240. <https://doi.org/10.1111/nmo.12822>.
- Panasevich MR, Morris EM, Chintapalli SV, Wankhade UD, Shankar K, Britton SL, Koch LG, Thyfault JP, Rector RS. 2016. Gut microbiota are linked to increased susceptibility to hepatic steatosis in low-aerobic-capacity rats fed an acute high-fat diet. *Am J Physiol Gastrointest Liver Physiol* 311:G166–G179. <https://doi.org/10.1152/ajpgi.00065.2016>.
- den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud D-J, Bakker BM. 2013. The role of short-chain fatty acids in the interplay between diet, gut microbiota and host energy metabolism. *J Lipid Res* 54:2325–2340. <https://doi.org/10.1194/jlr.R036012>.
- Al-Lahham SH, Peppelenbosch MP, Roelofsens H, Vonk RJ, Venema K. 2010. Biological effects of propionic acid in humans; metabolism, potential applications and underlying mechanisms. *Biochim Biophys Acta* 1801:1175–1183. <https://doi.org/10.1016/j.bbali.2010.07.007>.
- Cannon WB. 1929. Organization for physiological homeostasis. *Physiol Rev* 9:399–431. <https://doi.org/10.1152/physrev.1929.9.3.399>.
- Bhutia YD, Ganapathy V. 2015. Short, but smart: SCFAs train T cells in the gut to fight autoimmunity in the brain. *Immunity* 43:629–631. <https://doi.org/10.1016/j.immuni.2015.09.014>.
- Sun M, Wu W, Liu Z, Cong Y. 2017. Microbiota metabolite short chain fatty acids, GPCR, and inflammatory bowel diseases. *J Gastroenterol* 52:1–8. <https://doi.org/10.1007/s00535-016-1242-9>.
- Kimura I, Inoue D, Hirano K, Tsujimoto G. 2014. The SCFA receptor GPR43 and energy metabolism. *Front Endocrinol (Lausanne)* 5:85. <https://doi.org/10.3389/fendo.2014.00085>.
- Priyadarshini M, Wicksteed B, Schiltz GE, Gilchrist A, Layden BT. 2016. SCFA receptors in pancreatic  $\beta$  cells: novel diabetes targets? *Trends Endocrinol Metab* 27:653–664. <https://doi.org/10.1016/j.tem.2016.03.011>.
- Gerber P, Anzenberger G, Schnell CR. 2002. Behavioral and cardiophysiological responses of common marmosets (*Callithrix jacchus*) to social and environmental changes. *Primates* 43:201–216. <https://doi.org/10.1007/BF02629648>.
- Hartzell AL, Maldonado-Gómez MX, Hutkins RW, Rose DJ. 2013. Synthesis and in vitro digestion and fermentation of acylated inulin. *Bioact Carbohydr Diet Fibre* 1:81–88. <https://doi.org/10.1016/j.bcdf.2013.01.004>.
- Yamaoka K, Nakagawa T, Uno T. 1978. Application of Akaike's information criterion (AIC) in the evaluation of linear pharmacokinetic equations. *J Pharmacokin Biopharm* 6:165–175. <https://doi.org/10.1007/BF01117450>.
- Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Series B Methodol* 57:289–300. <https://doi.org/10.1111/j.2517-6161.1995.tb02031.x>.
- Ihaka R, Gentleman R. 1996. R: a language for data analysis and graphics. *J Comput Graph Stat* 5:299–314. <https://doi.org/10.2307/1390807>.