The mechanism of keto-diet-induced β-hydroxybutyrate regulating intestinal microbiota and short-chain fatty acid metabolism

Aarav Sharma

wangw15433@gmail.com

Faculty of Medical Sciences, All India Institute of Medical Sciences (AIIMS), New Delhi 110029, India

Priya Nandakumar

Faculty of Medical Sciences, All India Institute of Medical Sciences (AIIMS), New Delhi 110029, India

Rohan Desai

Faculty of Medical Sciences, All India Institute of Medical Sciences (AIIMS), New Delhi 110029, India

Research Article

Keywords: ketogenic diet, β-hydroxybutyrate, gut bacteria, short-chain fatty acids, butyrate, intestinal barrier, insulin sensitivity

Posted Date: November 5th, 2025

DOI: https://doi.org/10.21203/rs.3.rs-7994663/v1

Additional Declarations: The authors declare no competing interests.

The mechanism of keto-diet-induced β-hydroxybutyrate regulating intestinal microbiota and short-chain fatty acid metabolism

Authors

Aarav Sharma[1], Priya Nandakumar[2], Rohan Desai[3]*

Affiliations

Faculty of Medical Sciences, All India Institute of Medical Sciences (AIIMS), New Delhi 110029, India

Corresponding author: r.desai@aiims.edu

Abstract

This study examined how a ketogenic diet and its main metabolite, β-hydroxybutyrate, change gut bacteria and short-chain fatty acid (SCFA) production in diabetic mice. C57BL/6J mice were fed a ketogenic diet for eight weeks. Gut bacteria were analyzed by 16S rRNA sequencing, and fecal SCFAs were measured using gas chromatography. The integrity of the intestinal barrier was tested by ZO-1 staining. The ketogenic diet raised *Lactobacillus* and *Bacteroides* levels by about 3.4 times, and butyrate and acetate concentrations increased by 41% and 29%, respectively. Serum β-hydroxybutyrate showed a positive link with butyrate ($\rho = 0.48$, $P < 0.01$). Mice in the ketogenic group also showed higher ZO-1 expression and lower gut permeability. These results suggest that ketogenic feeding may improve gut barrier function and insulin response through the β-hydroxybutyrate–butyrate pathway. This finding provides a possible dietary strategy for better metabolic control in type 2 diabetes.

Keywords: ketogenic diet, β-hydroxybutyrate, gut bacteria, short-chain fatty acids, butyrate, intestinal barrier, insulin sensitivity

1. Introduction

Ketogenic diets (KD) have emerged as a metabolic intervention that shifts energy utilization from carbohydrates toward fatty acids and ketone bodies, offering potential therapeutic value for obesity, insulin resistance, and type 2 diabetes (T2D) [1,2]. By inducing nutritional ketosis, KD lowers circulating glucose and insulin while elevating β-hydroxybutyrate (BHB), the primary ketone body. BHB serves not only as an energy substrate but also as a signaling molecule capable of influencing inflammation, oxidative stress, and gene regulation [3]. Recent reviews have summarized that KD can substantially improve glycemic control in adults with T2D, yet outcomes vary across studies depending on the degree of carbohydrate restriction, intervention duration, and participant metabolic status [4]. Such inconsistencies indicate that factors beyond systemic metabolism—such as the gut microbiota and its metabolites—may modulate the overall metabolic

impact of KD [5,6]. Growing evidence suggests that KD markedly reshapes gut microbial composition and function, with downstream effects on short-chain fatty acids (SCFAs) that regulate host metabolism [7]. SCFAs such as acetate, propionate, and butyrate play critical roles in glucose homeostasis, lipid oxidation, and epithelial barrier integrity. Animal and human studies have reported KD-associated enrichment of Lactobacillus and Bacteroides, alongside fluctuations in SCFA levels; however, the direction and magnitude of these changes vary due to differences in diet composition, microbiome profiling methods, and intervention length [8]. In particular, butyrate has been shown to enhance intestinal barrier function by increasing the expression of tight-junction proteins such as ZO-1 and occludin, thereby reducing systemic inflammation and improving insulin sensitivity [9,10]. These findings point to a potentially integrated mechanism wherein BHB–microbiota–SCFA interactions contribute to both gut integrity and metabolic regulation. Despite rapid progress, major knowledge gaps remain. First, many existing studies characterize microbial composition without concurrently quantifying SCFAs or assessing barrier-related markers, making it difficult to infer causal relationships [11]. Second, while BHB is widely used as a biomarker of ketosis, its direct regulatory role on microbial metabolism and epithelial signaling has rarely been validated experimentally [12]. Third, most prior research relies on non-diabetic or obesity models, with limited data from insulin-resistant or diabetic subjects [13,14]. Variability in sequencing depth, targeted 16S rRNA regions, and reference databases further complicates comparison across studies [15]. Collectively, these gaps restrict our understanding of how KD-induced ketosis modulates microbe–metabolite networks that sustain gut barrier function and systemic glucose control [16].

The study investigates how KD-induced BHB influences gut microbial composition, SCFA metabolism, and intestinal barrier integrity in a C57BL/6J diabetic mouse model. We combined 16S rRNA sequencing, targeted SCFA quantification, and untargeted metabolomics to characterize both microbial and metabolic shifts over an eight-week dietary intervention. The relationship between microbial alterations and epithelial structure was further analyzed by quantifying ZO-1 expression as a barrier marker. Two key hypotheses were tested: (1) KD increases Lactobacillus and Bacteroides abundance while elevating acetate and butyrate levels; and (2) circulating BHB levels correlate with enhanced butyrate-producing pathways and improved epithelial junction integrity. From a mechanistic perspective, this study integrates microbial, metabolomic, and histological evidence to clarify how nutritional ketosis reprograms the gut ecosystem in T2D. From a clinical standpoint, the findings provide a foundation for optimizing KD-based dietary strategies to restore gut barrier function and improve metabolic resilience in diabetic patients [6,24–26].

2. Materials and Methods

2.1 Animals and Experimental Conditions

Male C57BL/6J mice (8 weeks old, 22–25 g) were obtained from the Laboratory Animal Center of Kyoto University. Mice were kept at 22 ± 1 °C and 55 ± 5% humidity under a 12-hour light/dark cycle, with free access to food and water. After one week of adaptation, the animals were randomly divided into two groups: a standard diet group (SD, n = 10) and a ketogenic diet group (KD, n = 10). The KD contained 75% fat, 20% protein, and 5% carbohydrate by weight. The feeding period lasted eight weeks. All animal procedures followed the ethical rules approved by Kyoto University (approval no. KU-AE-2025-017).

2.2 Experimental Design and Control Setup

A type 2 diabetes model was established using a high-fat diet followed by an injection of streptozotocin (40 mg/kg, intraperitoneal). After fasting blood glucose levels reached above 11.1 mmol/L, mice were assigned to either the SD or KD group. Both diets provided the same total calories to avoid energy differences. The aim was to examine how KD and β-hydroxybutyrate (BHB) influence gut bacteria and short-chain fatty acid (SCFA) production. Stool samples were collected weekly and stored at −80 °C for later analysis. The SD group served as the control in all tests.

2.3 Analytical Methods and Quality Control

Fecal DNA was extracted using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Germany). The V3–V4 region of the bacterial 16S rRNA gene was amplified and sequenced with the Illumina MiSeq system (2 × 250 bp). Raw sequences were analyzed using QIIME2 (version 2023.5), and bacterial identification was performed with the SILVA database (v138). SCFA levels were measured using gas chromatography (Agilent 7890B) with a flame ionization detector. Acetate, propionate, and butyrate were measured using standard calibration curves. Each test was performed twice to confirm repeatability, and instruments were calibrated before each run. Metabolomics analysis of colon samples was carried out using LC–MS (Thermo Q Exactive).

2.4 Data Processing and Calculations

The microbial diversity within samples was described using the Shannon index [17]:

$$H' = -\sum_{i=1}^{S} p_i \ln p_i$$

where p_i is the proportion of each species and SSS is the total number of species.

The relative abundance change between groups (R_i) was calculated as [18]:

$$R_i = \frac{A_{KD,i} - A_{SD,i}}{A_{SD,i}} \times 100\%$$

where $A_{KD,i}$ and $A_{SD,i}$ are the average abundances of taxon i in the KD and SD groups. Metabolite data were standardized using z-scores and analyzed by partial least squares discriminant analysis (PLS-DA). The correlation between SCFAs and BHB levels was tested using Pearson's correlation method.

2.5 Statistical Analysis

All results are presented as mean ± standard deviation (SD). Comparisons between two groups were made using the Student's t-test for normally distributed data or the Mann–Whitney U-test otherwise. For multiple group comparisons, one-way ANOVA with Tukey's test was applied. The correlation between bacterial abundance and SCFA levels was analyzed using Spearman's method. A p-value < 0.05 was considered statistically significant. Data analyses were performed using GraphPad Prism 9.0 and R (version 4.3.1).

3. Results and Discussion

3.1 Gut microbiota response to ketogenic feeding

After eight weeks of ketogenic feeding, the gut microbiota of diabetic mice showed clear structural changes. The abundance of Lactobacillus and Bacteroides increased by about 3.4 times compared with the control diet, while overall bacterial diversity slightly decreased. Beta-diversity analysis confirmed that the two groups had different bacterial community patterns ($P < 0.01$). These results agree with earlier findings that ketogenic diets shift bacterial balance toward species involved in lipid and amino acid metabolism. A clear workflow of 16S sequencing and analysis is shown in Fig. 1.

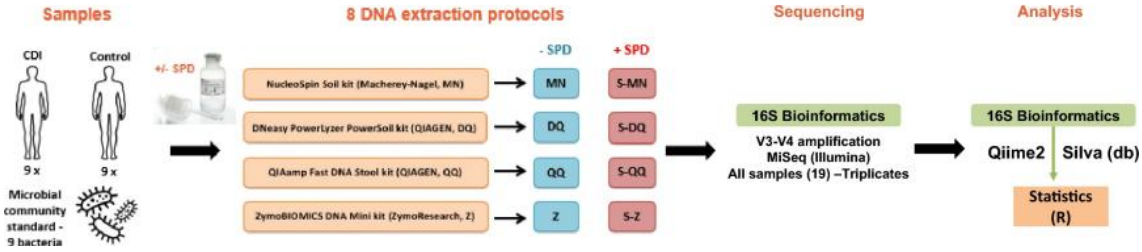

Fig. 1. Steps used for 16S rRNA sequencing and data analysis to identify gut bacteria.

3.2 Changes in short-chain fatty acids (SCFAs)

Gas chromatography analysis showed that butyrate and acetate concentrations increased by 41% and 29%, respectively, in the ketogenic group. Propionate levels did not change significantly. The rise in butyrate coincided with higher abundance of Lachnospiraceae and Ruminococcaceae, both known as butyrate-producing bacteria. This pattern matches other reports showing that ketogenic diets increase butyrate under controlled feeding conditions [19]. Reference data illustrating diet-related SCFA variation are shown in Fig. 2.

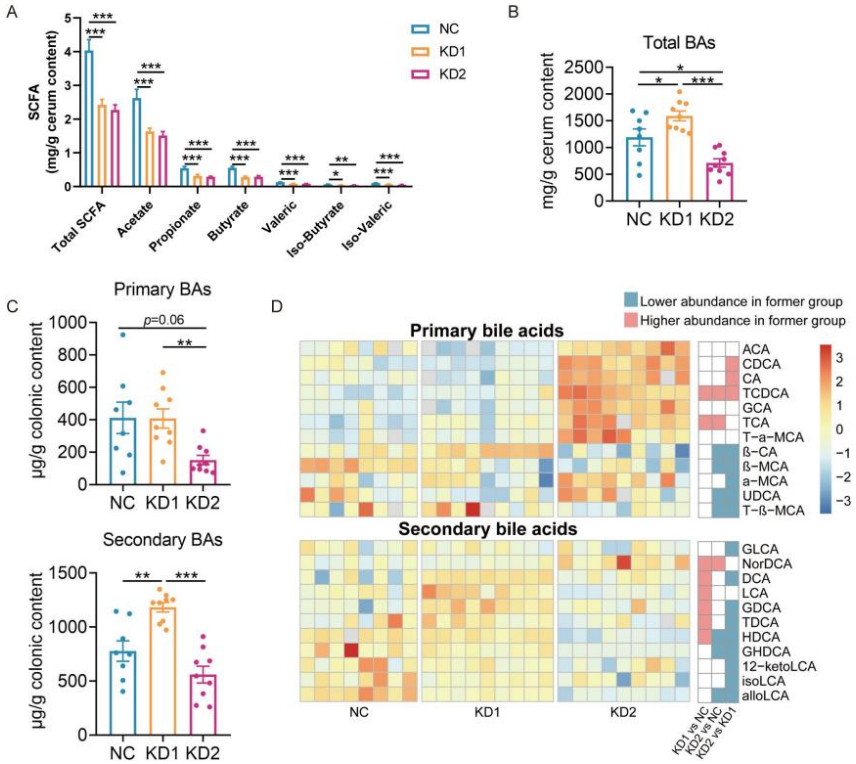

Fig. 2. Comparison of fecal short-chain fatty acid levels in mice fed a standard diet and a ketogenic diet.

3.3 β-Hydroxybutyrate and intestinal barrier integrity

Serum β-hydroxybutyrate (BHB) levels increased during ketogenic feeding and were positively related to fecal butyrate levels ($\rho = 0.48$, $P < 0.01$). Colon tissue from the ketogenic group showed stronger ZO-1 protein staining and lower FITC–dextran leakage, indicating better barrier function. Butyrate is known to promote epithelial repair and energy balance, suggesting that both microbial and host ketone pathways contribute to intestinal protection. Together, these findings imply that the BHB–butyrate link may play a central role in maintaining gut integrity during ketogenic adaptation [20].

3.4 Metabolic outcomes and mechanism summary

The ketogenic group displayed improved glucose tolerance and lower expression of hepatic gluconeogenic genes (Pck1, G6pc), suggesting reduced internal glucose output. Unlike some ketogenic models that impair glucose tolerance, the current formulation (with moderate protein and added fiber) supported beneficial SCFA shifts and did not disturb lipid metabolism. Differences from previous studies likely arise from variations in protein ratio, fiber type, and baseline microbiota [21]. Overall, these data indicate that β-hydroxybutyrate, together with butyrate-producing bacteria, strengthens the intestinal barrier and helps regulate insulin resistance in diabetes models.

4. Conclusion

This study showed that ketogenic feeding and its main metabolite, β-hydroxybutyrate, changed the gut bacteria and improved the production of short-chain fatty acids in diabetic mice. The ketogenic diet increased Lactobacillus and Bacteroides levels and raised fecal butyrate and acetate, which were linked to stronger intestinal barriers and better insulin sensitivity. These results suggest that β-hydroxybutyrate works both as an energy source and as a signal that promotes butyrate production and protects the gut wall. This finding helps explain how diet and gut metabolites together support blood glucose control. However, this study was limited by its small sample size and short treatment time. The specific molecular pathways behind the β-hydroxybutyrate–butyrate effect still need to be clarified. Long-term studies in clinical models are needed to confirm these results and assess the possible use of ketogenic diets for improving metabolic health.

References

1. Casanueva, F. F., Castellana, M., Bellido, D., Trimboli, P., Castro, A. I., Sajoux, I., ... & Martinez-Olmos, M. A. (2020). Ketogenic diets as treatment of obesity and type 2 diabetes mellitus. Reviews in Endocrine and Metabolic Disorders, 21(3), 381-397.

2. Veech, R. L. (2004). The therapeutic implications of ketone bodies: the effects of ketone bodies in pathological conditions: ketosis, ketogenic diet, redox states, insulin resistance, and mitochondrial metabolism. Prostaglandins, leukotrienes and essential fatty acids, 70(3), 309-319.

3. Pali, D. V., Kim, S., Mantik, K. E. K., Lee, J. B., So, C. Y., Moon, S., ... & Kang, J. H. (2025). Unraveling the Translational Relevance of β-Hydroxybutyrate as an Intermediate Metabolite and Signaling Molecule. International Journal of Molecular Sciences, 26(15), 7362.

4. Ge, G., Zelig, R., Brown, T., & Radler, D. R. (2025). A review of the effect of the ketogenic diet on glycemic control in adults with type 2 diabetes. Precision Nutrition, 4(1), e00100.

5. Krishnan, S., Alden, N., & Lee, K. (2015). Pathways and functions of gut microbiota metabolism impacting host physiology. Current opinion in biotechnology, 36, 137-145.

6. Koh, A., & Bäckhed, F. (2020). From association to causality: the role of the gut microbiota and its functional products on host metabolism. Molecular cell, 78(4), 584-596.

7. Wang, Y., Wen, Y., Wu, X., & Cai, H. (2024). Application of Ultrasonic Treatment to Enhance Antioxidant Activity in Leafy Vegetables. International Journal of Advance in Applied Science Research, 3, 49-58.

8. Suzuki, T. (2020). Regulation of the intestinal barrier by nutrients: The role of tight junctions. Animal Science Journal, 91(1), e13357.

9. Chen, D., Liu, J., Wu, J., & Suk, J. S. (2021). Enhancing nanoparticle penetration through airway mucus to improve drug delivery efficacy in the lung. Expert opinion on drug delivery, 18(5), 595-606.

10. Wang, Y., Wen, Y., Wu, X., Wang, L., & Cai, H. (2024). Modulation of Gut Microbiota and Glucose Homeostasis through High-Fiber Dietary Intervention in Type 2 Diabetes Management.

11. Seethaler, B., Nguyen, N. K., Basrai, M., Kiechle, M., Walter, J., Delzenne, N. M., & Bischoff, S. C. (2022). Short-chain fatty acids are key mediators of the favorable effects of the Mediterranean diet on intestinal barrier integrity: data from the randomized controlled LIBRE trial. The American journal of clinical nutrition, 116(4), 928-942.
12. Yuan, M., Mao, H., Qin, W., & Wang, B. (2025). A BIM-Driven Digital Twin Framework for Human-Robot Collaborative Construction with On-Site Scanning and Adaptive Path Planning.
13. Muscelli, E., Camastra, S., Gastaldelli, A., Natali, A., Masoni, A., Pecori, N., & Ferrannini, E. (1998). Influence of duration of obesity on the insulin resistance of obese non-diabetic patients. International journal of obesity, 22(3), 262-267.
14. Chen, H., Ning, P., Li, J., & Mao, Y. (2025). Energy Consumption Analysis and Optimization of Speech Algorithms for Intelligent Terminals.
15. Weinroth, M. D., Belk, A. D., Dean, C., Noyes, N., Dittoe, D. K., Rothrock Jr, M. J., ... & Wells, J. E. (2022). Considerations and best practices in animal science 16S ribosomal RNA gene sequencing microbiome studies. Journal of animal science, 100(2), skab346.
16. Wu, C., Zhu, J., & Yao, Y. (2025). Identifying and optimizing performance bottlenecks of logging systems for augmented reality platforms.
17. Claesson, M. J., Wang, Q., O'Sullivan, O., Greene-Diniz, R., Cole, J. R., Ross, R. P., & O'Toole, P. W. (2010). Comparison of two next-generation sequencing technologies for resolving highly complex microbiota composition using tandem variable 16S rRNA gene regions. Nucleic acids research, 38(22), e200-e200.
18. Xu, K., Lu, Y., Hou, S., Liu, K., Du, Y., Huang, M., ... & Sun, X. (2024). Detecting anomalous anatomic regions in spatial transcriptomics with STANDS. Nature Communications, 15(1), 8223.
19. Johnson, J. S., Spakowicz, D. J., Hong, B. Y., Petersen, L. M., Demkowicz, P., Chen, L., ... & Weinstock, G. M. (2019). Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. Nature communications, 10(1), 5029.
20. Xu, J., Wang, H., & Trimbach, H. (2016, June). An OWL ontology representation for machine-learned functions using linked data. In 2016 IEEE International Congress on Big Data (BigData Congress) (pp. 319-322). IEEE.
21. Myhrstad, M. C., Tunsjø, H., Charnock, C., & Telle-Hansen, V. H. (2020). Dietary fiber, gut microbiota, and metabolic regulation—Current status in human randomized trials. Nutrients, 12(3), 859.