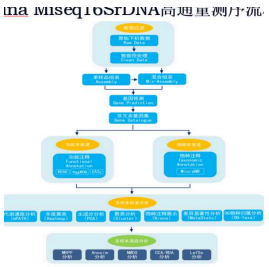
Nov 26, 2024

Changes in intestinal mucosal barrier function in obese mice



DOI
https://dx.doi.org/10.17504/protocols.io.bp2l6dbw5vqe/v1

xiaolin Gao[1], Mingli Guan[1], Qiuju Li[1], Ruizhen Jia[1], Jianjun Deng[1]

[1]West China Second Hospital

xiaolin Gao: 1
Mingli Guan: 2
Qiuju Li: 3
Ruizhen Jia: 4
Jianjun Deng: 5

xiaolin Gao
West China Second Hospital

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.bp2l6dbw5vqe/v1

Protocol Citation: xiaolin Gao, Mingli Guan, Qiuju Li, Ruizhen Jia, Jianjun Deng 2024. Changes in intestinal mucosal barrier function in obese mice. **protocols.io https://dx.doi.org/10.17504/protocols.io.bp2l6dbw5vqe/v1**

Protocol status: Working
We use this protocol and it's working

Created: November 26, 2024

Last Modified: November 26, 2024

Protocol Integer ID: 112772

Keywords: Intestinal mucosal barrier Function, Obesity, Mice, intestinal mucosal barrier in obese patient, changes in intestinal mucosal barrier function, intestinal mucosal barrier function, intestinal mucosal barrier, characteristics of intestinal flora, intestinal flora, intestinal microbiota phyla, morphological characteristics of the ileal mucosa, ileal mucosa of the hfd group, ileal mucosa, obese mice introduction few report, obese mice introduction, obese mice, intestinal gland, epithelial shedding at the intestinal villus tip, faece, intestinal villus tip, plasma diamine oxidase, endotoxin levels in the hfd group, fat diet, endotoxin level

Disclaimer

The authors have no financial relationships relevant to this article to disclose.

Abstract

Introduction Few reports have described changes in other functions of the intestinal mucosal barrier in obese patients worldwide. **Objectives** To observe the changes in intestinal mucosal barrier function in obese mice. **Methods** Fifty healthy male C57BL/6J mice were randomly divided into a high-fat diet (HFD)-induced obese group and a control group (CON), with 25 mice in each group. Plasma diamine oxidase (DAO), D-lactate and endotoxin levels were detected via the enzyme-labelled colorimetric method. Characteristics of intestinal flora in faeces were detected via Illumina HiSeq 16S rDNA high-throughput sequencing technology. The morphological characteristics of the ileal mucosa were observed via optical microscopy. The data were analysed via bioinformatics and statistics. **Results** The plasma levels of DAO, D-lactate, and endotoxin levels in the HFD group were significantly higher than those in the CON group at week 10（$P<0.05$）. The plasma DAO, D-lactate, and endotoxin levels in the HFD group were significantly greater than in the CON group ($P<0.05$). The CON group contained more intestinal microbiota phyla and genera than the HFD group; the differences between the two groups were significant（$FDR\leq0.05$, $P\leq0.05$). The ileal mucosa of the HFD group exhibited more epithelial shedding at the intestinal villus tip, and the intestinal glands were more abundant. **Conclusion** This study is expected to provide a basic experimental reference for further research on the mechanism, prevention, and treatment of obesity.

Attachments

Fig.1.jpg 23KB
Fig.2.jpg 74KB
Fig.3.jpg 31KB
Fig.4.jpg 233KB
Fig.5.jpg 149KB

Image Attribution

Figure
1 OTU Venn Figure
Figure
2 Box-plot of α diversity of the obesity group and the control group
Figure
3 PCoA analysis of the obesity group and the control group based on OTU abundance
Figure
4 Histogram of the abundance distribution of phylum and genus of intestinal microbiota（Top15）
Figure
5 Results of HE staining of mouse ileal mucosal sections (Under an optical microscope, 40Times)

Guidelines

To ensure the accuracy of the results, precise declutter can be performed to remove sequences containing ambiguous bases, single-base high-repeating regions, and excessively short sequences. The parameters for precise decontamination are: the sequence containing N bases is removed, and the sequence with a base mass fraction Q20 of at least 75% is retained. At the same time, the chimeric sequences were detected and removed by UCHIME.
After the sequencing data were preprocessed to generate high-quality sequences, Vsearch[5] software was used to classify sequences into multiple OTUs according to their similarity. The argument that sequence similarity is greater than or equal to 97% is classified as an OTU cell.
The QIIME [6] package was used to pick out the representative sequences of each OTU and annotate all the representative sequences against the database. Silva (version138) database was used for comparison, and the RDP classifier [7] software was used for species comparison annotations, and the annotation results with confidence intervals greater than 0.7 were retained. 0 µL 0 µL 0 µL

Materials

Animals

Healthy C57BL/6J SPF-grade mice (male; 6 weeks old; body weight, 23–28g) with the diet purchased from Chengdu Quan Xi Biotechnology Co., Ltd. The mice were adaptively fed for 1 week before the experiment. Humidity was controlled within 50% to 70%, and the temperature was maintained between 20 to 24 °C, with a 12-hour light/dark cycle. The animals had free access to food and water.

The mice were randomly divided into 2 groups using the computer method, namely, the HFD group and the control group, with 25 mice in each group. The living environments of the two groups were identical, and the experimental observation period was 10 weeks. The HFD group was fed a high-fat diet (fat 60.0%, protein 19.4%, carbohydrate 20.6%; calories 5.0 kcal/g), and the CON group was fed a normal diet (fat 10.0%, protein 19.0%, carbohydrate 71.0%; calories 3.6 kcal/g).Two grams of fresh feces were collected at fixed time points in week 1 and week 10 respectively and stored in the -80 °C refrigerator. All the mice were euthanized by pentobarbital at week 10. Abdominal white adipose and ileum tissues were collected for examination. Blood samples were collected, centrifuged, and stored in a -20 °C freezer until analysis.

Safety warnings

Ethics statement

This study was approved by the Ethical Committee on Animal Experimentation of the Ethics Committee of West China Second Hospital, Sichuan University (NO.2020008). All methods were carried out in accordance with relevant guidelines and regulations.

16S rRNA amplicon sequencing

Protocol references

[1] Bajaj SS, Jain B, Kyle TK, *et al*.Overcoming congressional inertia on obesity requires better literacy in obesity science.OBESITY,2002, 30(4):799-801.
[2] Tahrani AA, Morton J.Benefits of weight loss of 10% or more in patients with overweight or obesity: A review.OBESITY, 2022, 30(4):802-840.
[3] Serrano-Fuentes N, Rogers Anne, Portillo MC. Beyond individual responsibility: Exploring lay understandings of the contribution of environments on personal trajectories of obesity. PLoS One, 2024, 19(5):e0302927.
[4] Gao X, Jia R, Xie L, *et al*. A study of the correlation between obesity and intestinal flora in school-age children. Sci Rep, 2018, 8(1):14511.
[5] Le Roy T, Moens de Hase E, Van Hul M, *et al*. Dysosmobacter welbionis is a newly isolated human commensal bacterium preventing diet-induced obesity and metabolic disorders in mice.GUT, 2022,71(3):534-543.
[6] Li QJ, Gao XL, Jia RZ, et al. Establishment of a novel obesity mouse model: the induction of intestinal microbiota dysbiosis. Sci Rep, 2024, 14(1):15950.
[7] Huang L, He F, Wu B. Mechanism of effects of nickel or nickel compounds on intestinal mucosal barrier. CHEMOSPHERE, 2022, 305:135429.
[8] An J, Liu Y, Wang Y, *et al*. The Role of Intestinal Mucosal Barrier in Autoimmune Disease: A Potential Target. Front Immunol, 2022,13: 871713.
[9] Bliesner A, Eccles-Smith J, Bates C, *et al*. Impact of Food-Based Weight Loss Interventions on Gut Microbiome in Individuals with Obesity: A Systematic Review.Nutrients. 2022-05-06; 14(9).
[10] Yang J, Xiong P, Bai L, *et al*. The Association of Altered Gut Microbiota and Intestinal Mucosal Barrier Integrity in Mice With Heroin Dependence.Front Nutr, 2021, 8:765414.
[11] Gao X, Miao R, Tao Y, *et al*. Effect of Montmorillonite powder on intestinal mucosal barrier in children with abdominal Henoch-Schonlein purpura: A randomized controlled study. MEDICINE, 2018, 97(39):e12577.
[12] Chen S, Xiu G, Zhou J, *et al*. Role of high mobility group box 1 in intestinal mucosal barrier injury in rat with sepsis induced by endotoxin. Zhonghua Wei Zhong Bing Ji Jiu Yi Xue, 2020, 32(7):803-807.
[13] Dieryck I, De Backere J, Paeshuyse J. Effect of hatching system and prophylactic antibiotic use on serum levels of intestinal

health biomarker diamine oxidase in broilers at an early age. ANIMAL, 2022, 16(4):100493.
[14] Zhang X, Liu D, Wang Y, *et al*. Clinical significance on serum intestinal fatty acid binding protein and D-lactic acid levels in early intestinal injury of patients with sepsis.Zhonghua Wei Zhong Bing Ji Jiu Yi Xue, 2019,31(5):545-550.
[15] Huang J, Guan B, Lin L, et al. Improvement of intestinal barrier function, gut microbiota, and metabolic endotoxemia in type 2 diabetes rats by curcumin.BIOENGINEERED, 2021, 12(2):11947-11958.

Acknowledgements

The authors would like to thank all study subjects and the staff involved in this study.