BKS-db

Strain Name: BKS-*Lepr*^{em2Cd479}/Gpt
Strain Number: T002407
Strain Type: Cas9-KO
Background: C57BLKS/JGpt

Description

The Obese Gene Receptor (ob-R) also known as LEPR gene is encoding a Type I cytokine receptor, a protein that in humans have a large relationship with obesity, hypertension, diabetes, lipid metabolism disorders, etc. [1,2]. The db/db mouse is a model of obesity, diabetes, and dyslipidemia wherein leptin receptor activity is deficient because leptin mutation are performed by CRISPR/Cas9 technology. The disease course is greatly affected by the genetic background, *Lepr* defects in the BKS background are often accompanied by higher levels of blood sugar and body weight. Higher levels of blood glucose and gluconeogenesis enzyme activity cannot be controlled by insulin treatment on BKS-db mouse, in which accompanied by phenotypes of peripheral neuropathy, myocardial disease, delayed wound healing, accelerated metabolic efficiency [3], hypothalamic lesions [4]. In addition, female db/db mice were observed infertility together with reduced uterine and ovarian weight and decreased estrogen secretion [5-6].
The *Lepr* gene mutant mouse (BKS-db) was constructed by GemPharmatech Co., Ltd using gene editing technology. By monitoring the blood glucose levels for this strain, it was found that BKS-db mouse have significantly higher level of blood glucose than that of the wild control. This strain is an ideal model for type II diabetes research.

Application

1. Metabolic research (diabetes and obesity);
2. Endocrine Disorder research;
3. Reproductive Biology research.

Data support

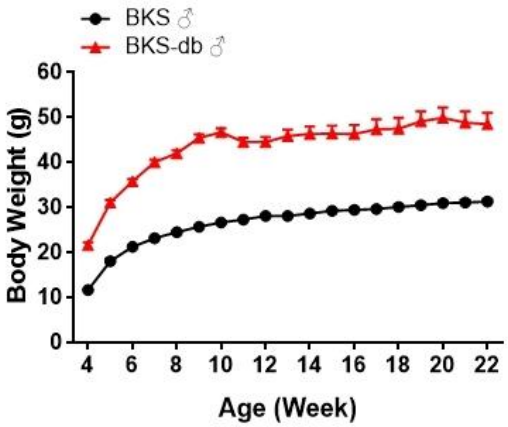

Fig 1. Weight gain in BKS-db mice

BKS-db mice rapidly gained weight after 4 weeks of age. (Data were presented as Mean±SEM, n=20.)

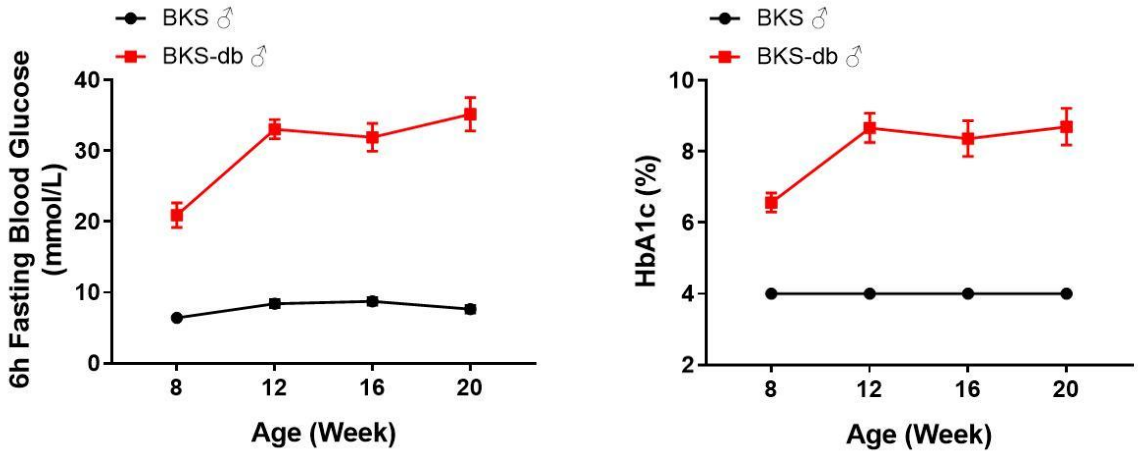

Fig 2. Increased blood glucose levels in BKS-db mice

When fasting blood glucose was measured in mice fasted for 6 h, BKS-db was significantly increased compared with the control, and remained stable after 8 weeks of age. (The data were shown as Mean± SEM, n=5~6. **The data is only the test value of the sample in this experiment. The actual blood glucose fluctuation range of mice between individuals is wide, and the test results may be biased**.)

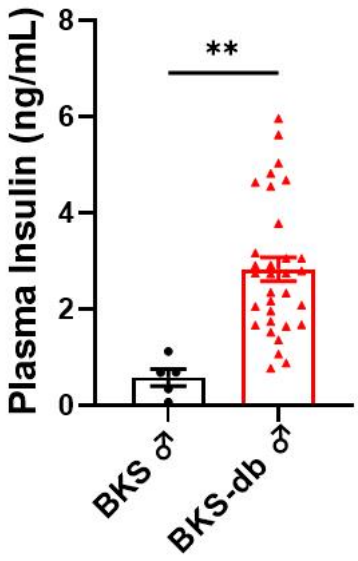

Fig 3. Increased insulin levels in BKS-db mice

The blood insulin level of 8-week-old BKS-db mice was significantly higher than that of control mice. (Data were presented as Mean±SEM, n=6~32. Statistical analysis was performed using unpaired two-tailed Student's t-test method. ****, P<0.0001 compared with CD group.)

Note: The detection limits and sensitivities of insulin Elisa kits from different manufacturers are different, so the absolute value of insulin may be variable when using the kit from different suppliers. The current data come from the kit provided by crystalchem (catalog number 90080).

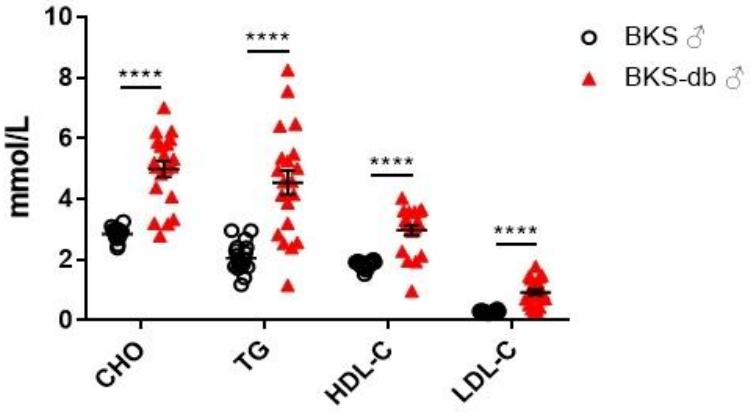

Fig 4. Increased lipid levels in BKS-db mice

The blood levels of four lipids (triglyceride, cholesterol, low-density lipoprotein cholesterol and high-density lipoprotein cholesterol) in 8-week-old BKS-db mice were significantly higher than those in controls. (Data were presented as Mean±SEM, n=20. Statistical analysis was performed using the unpaired two-tailed Student's t-test method. ****, P<0.0001 compared to the CD group.)

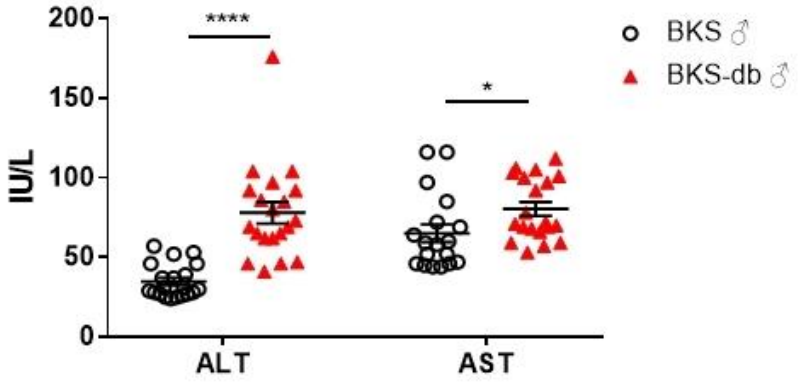

Fig 5.Liver damage in BKS-db mice

The levels of alanine aminotransferase and aspartate aminotransferase in the blood of 8-week-old BKS-db mice were significantly higher than those of the control. (Data were presented as Mean±SEM, n=20. Statistical analysis was performed using unpaired two-tailed Student's t-test method. *, P<0.05; ****, P<0.0001 compared to CD group.)

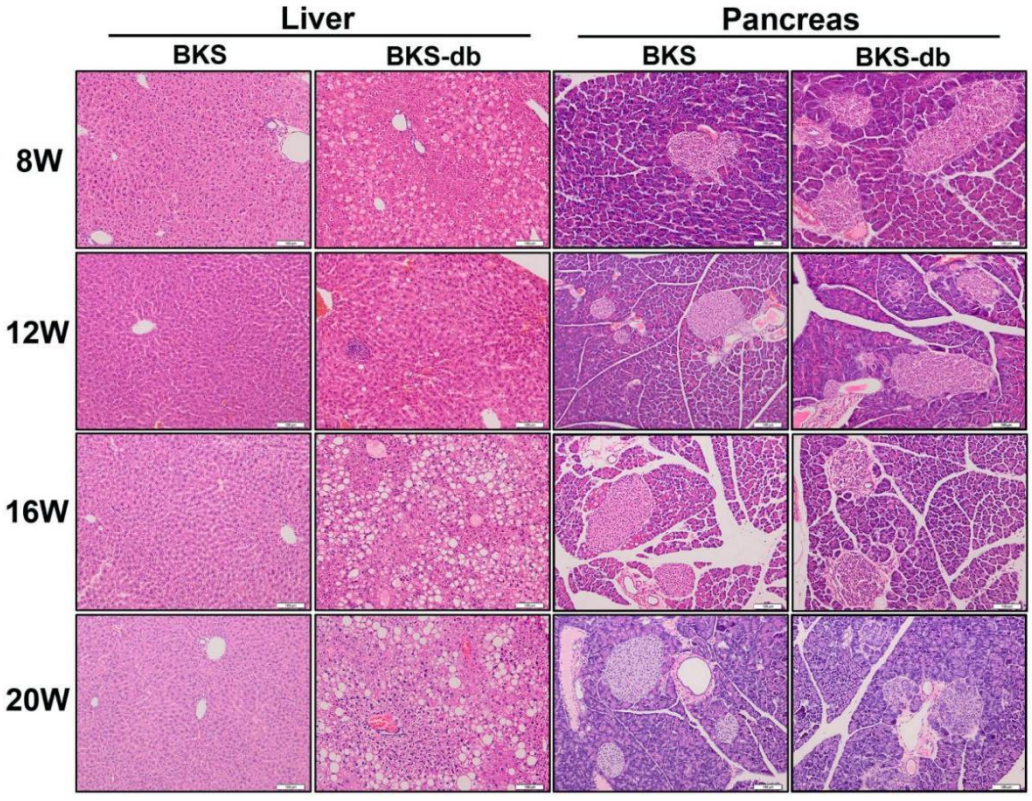

Fig 6. Liver and pancreas lesions in BKS-db mice

BKS-db mice showed marked steatosis in the liver after 12 weeks of age, and the islet tissue in the pancreas was disorganized.

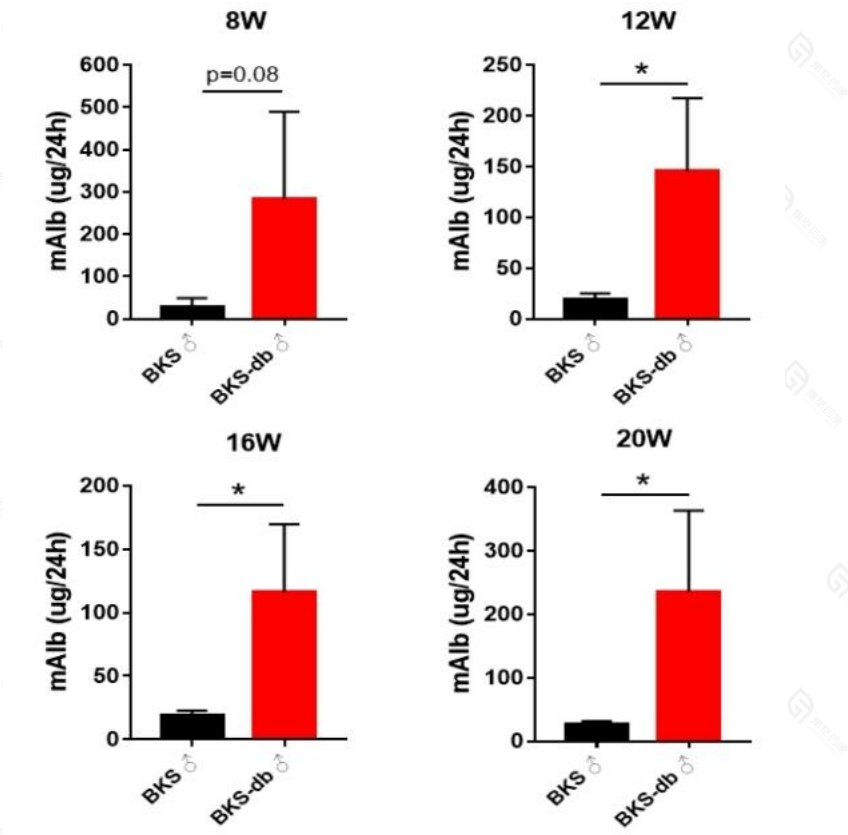

Fig 7. Early symptoms of diabetic nephropathy in BKS-db mice

The level of mAlb in urine of BKS-db mice was significantly increased after 12 weeks of age. (The data were presented as Mean±SD, n=5~6. Statistical analysis was performed using the unpaired two-tailed Student's t-test method. *, P<0.05 compared with the CD group. **Note: This data was collected by collecting 24h urine , measured by blood biochemical method, possible impurities in urine, different detection methods, and the number of enrolled mice may lead to deviations in the results. This data is for reference only, and individual differences are not excluded.**)

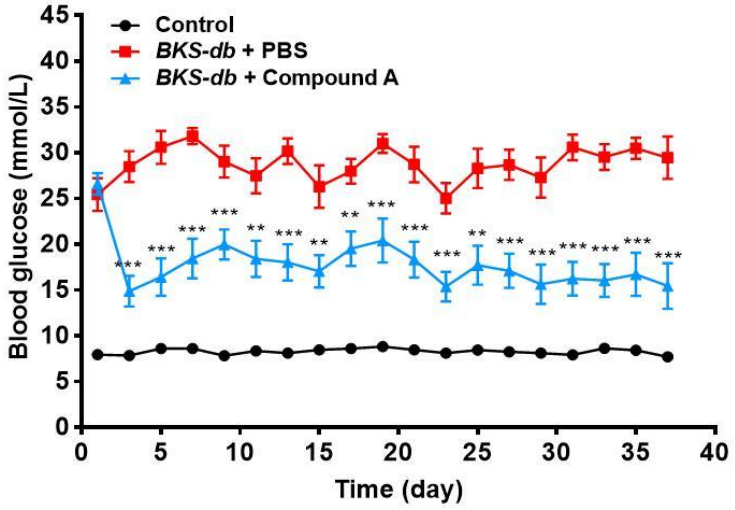

Fig 8. Effects of drug intervention on blood glucose in BKS-db mice

The blood glucose of BKS-db diabetic mice was significantly decreased under the intervention of the tested drugs. (Control group n = 14, BKS-db + PBS group n = 12, BKS-db + Compound A group n = 12. Data were presented as Mean ± SEM, using one-way ANOVA with Dunnett post-hoc test method for statistics Biological analysis. **, P<0.01; ***, P<0.001 compared with BKS-db + PBS group.)

Precautions

1. Characteristics of blood glucose

BKS-db is a period of rapid blood glucose rise at 4-8 weeks of age. During this period, the blood glucose of mice has a wide range, and the blood glucose between different individuals is also very different. Blood glucose groupings were strictly measured before, and the experimental results may have certain errors. After 8 weeks of age, the blood glucose levels gradually tend to be consistent, but there are still individual differences that lead to uneven blood glucose. It is recommended to increase the margin by 20% to 30%. Onset criteria: fasting blood glucose>11.1mmol/l, random blood glucose>16.7mmol/l[7-8]. Environmental stimuli, mouse state, measurement time, and satiety (fasting time) can all affect blood glucose measurements. It is recommended to be raised in a barrier facility, ensure adequate drinking water and diet, change bedding frequently, reduce stress, and unify the time and blood collection method for blood glucose measurement. After receiving the mice, adaptive feeding was performed for 1 to 2 weeks for the experiment. Fasting blood sugar fasting generally does not exceed 6h.

2. Characteristics of diabetic nephropathy

Under normal circumstances, some mice began to develop severe renal complications at the age of 16 weeks, manifested as adhesion of renal tissue, unable to remove the intact kidney for pathological examination or death of the mouse. Some mice with higher blood glucose may develop similar severe kidney complications around 12 weeks of

age. For blood glucose-related research, it is recommended to add an additional 20% to 30% of the surplus on the basis of the actual number of participants, or to select materials from an earlier age. Generally, the evaluation indicators for renal lesions include pathological sections, blood biochemistry and urine biochemistry, among which the urine biochemistry data is the most unstable, and it is recommended to combine the other two tests for judgment. For studies related to diabetic nephropathy, it is necessary to strictly set the entry indicators and select appropriate mice for the experiment. In addition, batch-to-batch differences are prone to occur, and intra-batch comparisons are recommended.

3. Feeding related

BKS-db homozygous mice, the phenotype can be seen at approximately three weeks of age. Homozygous mice gain weight rapidly, up to three times the normal weight of wild-type control mice. Homozygous mice are obese, with enlarged buttocks, wider legs, and short legs. Homozygous male mice are prone to genital atrophy over 8 weeks of age. This strain is a diabetic model mouse. With age, blood glucose and body weight will gradually increase. The blood glucose of 5-9 weeks old mice will increase rapidly, and it will reach a critical value after 9 weeks of age. Later, mice will lose weight, etc. Diabetic complication phenotype, the body weight of mice of the same age varies greatly. Note that 5-9 weeks of age is the period of rising blood glucose and body weight of mice. During this period, the mouse cage is more likely to be dirty and needs to be changed once a week, and twice a week. The blood glucose will reach the critical value around 9 weeks of age. Rat cage boxes are particularly prone to moisture, and often need to be replaced every two days, and even some older stock cages need to be replaced once a day. Therefore, in the case of sufficient cage space, it is necessary to reduce the cage density of older stocks.

References

1. Chen, Hong, et al. "Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice." Cell 84.3 (1996): 491-495.
2. Yoon, Ji-Won, et al. "Genetic control of organ-reactive autoantibody production in mice by obesity (ob) diabetes (db) genes." Diabetes 37.9 (1988): 1287-1293.
3. Kämpfer, Heiko, et al. "Lack of interferon-gamma production despite the presence of interleukin-18 during cutaneous wound healing." Molecular Medicine 6.12 (2000): 1016.
4. Garris, Bryan L., et al. "Hypophyseal lipoapoptosis: diabetes (db/db) mutation-associated cytolipidemia promotes pituitary cellular disruption and dysfunction." Pituitary 7.1 (2004): 5-14.
5. Garris, David R. "Estrogenic stimulation of ovarian follicular maturation in diabetes

(db/db) mutant mice: restoration of euglycemia prevents hyperlipidemic cytoatrophy." Cell and tissue research 318.2 (2004): 365-373.

6. Garris, David R., and Bryan L. Garris. "Genomic modulation of diabetes (db/db) and obese (ob/ob) mutation-induced hypercytolipidemia: cytochemical basis of female reproductive tract involution." Cell and tissue research 316.2 (2004): 233-241.
7. Edirs S, Jiang L, Lei X, et al. "XinKursi Wufarikun Ziyabit Improves the Physiological Changes by Regulating Endoplasmic Reticulum Stress in the Type 2 Diabetes db/db Mice." Evid Based Complement Alternat Med. 2021 Aug 16;2021:2100128.
8. Huang CZ, Xu JH, Zhong W, et al. "Sox9 transcriptionally regulates Wnt signaling in intestinal epithelial stem cells in hypomethylated crypts in the diabetic state. " Stem Cell Res Ther. 2017 Mar 9;8(1):60.