# Protocol

Evaluation of AMPK activity in mice by measuring activator-induced glucose uptake

| Day 1 | 1) Fast mice (12~16hr) |
|-------|------------------------|
|       | 2.1) Weigh each mouse and calculate the injection volume |
|       | 2.2) Cut 1~2 mm of the tail and record the glucose value measured by the glucometer (t = 0) |
|       | 2.3) Inject mice with MK8722 (30mg/kg) or A769662 (60mg/kg) solution |
|       | 2.4) Measure blood glucose at 15, 30, 45, 60, 90 and 120 minutes after injection. |

The AMP-activated protein kinase (AMPK) is a principal nutrient sensor and a master regulator of cellular energy homeostasis. Once activated, AMPK induces glucose uptake, which leads to a transient decrease in blood glucose level and can be used as an indicator of AMPK activity. Here, we present a protocol accessing AMPK activity in mice by measuring glucose uptake induced by AMPK activators, MK8722 and A769662. This protocol can be used to evaluate AMPK signaling in vivo under various pathophysiological conditions.

**Highlights**
- Measurement of blood glucose level in mice using glucometer
- Inducing glucose uptake in mice by administration of AMPK activators
- Evaluation of AMPK activity in vivo via measuring AMPK activator-induced glucose

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Peng Jiang, Li Zhi, Lejiao Ren, Xinli Hu, Rui-Ping Xiao
huwx025@pku.edu.cn (X.H.)
xiaor@pku.edu.cn (R.-P.X.)

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Protocol
Evaluation of AMPK activity in mice by measuring activator-induced glucose uptake

Peng Jiang,1,3 Li Zhi,2 Lejiao Ren,2 Xinli Hu,1,* and Rui-Ping Xiao1,2,4,*

1State Key Laboratory of Membrane Biology, Institute of Molecular Medicine, College of Future Technology, Peking University, Beijing 100871, China
2Peking-Tsinghua Center for Life Sciences, Academy for Advanced Interdisciplinary Studies, Peking University, Beijing 100871, China
3Technical contact
4Lead contact
*Correspondence: huxxx025@pku.edu.cn (X.H.), xiaor@pku.edu.cn (R.-P.X.)
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SUMMARY
The AMP-activated protein kinase (AMPK) is a principal nutrient sensor and a master regulator of cellular energy homeostasis. Once activated, AMPK induces glucose uptake, which leads to a transient decrease in blood glucose level and can be used as an indicator of AMPK activity. Here, we present a protocol accessing AMPK activity in mice by measuring glucose uptake induced by AMPK activators, MK8722 and A769662. This protocol can be used to evaluate AMPK signaling in vivo under various pathophysiological conditions. For complete details on the use and execution of this protocol, please refer to Jiang et al. (2021).

BEFORE YOU BEGIN
AMPK activation induces glucose uptake, especially in skeletal muscle (Musi and Goodyear, 2003). Measurement of glucose uptake stimulated by AMPK activators (Myers et al., 2017; Wu et al., 2013) can serve as a proxy of AMPK activity in vivo.

Preparation of MK8722 solution

Timing: 10 min
1. Buffer for MK8722: 10% DMSO (v/v), 40% PEG300 (v/v), 5% Tween 80 (v/v) and 45% saline (v/v).
2. Sterilize the buffer by passing through a 0.22 μM filter.
3. Dissolve MK8722 in the above buffer to a final concentration of 3 mg/mL.

Note: Freshly prepare the solution before each use.

4. The dosage is 30 mg/kg (Myers et al., 2017). Using a MK8722 stock solution of 3 mg/mL, the volume of intraperitoneal injection into mouse = BW (g) × 10 μL/g BW.

Preparation of A769662 solution

Timing: 10 min
5. Buffer for A769662: 5% DMSO (v/v), 40% PEG300 (v/v), 5% Tween 80 (v/v) and 50% saline (v/v).
6. Sterilize the buffer with a 0.22 μM filter.
7. Dissolve A769662 in the above buffer to a final concentration of 6 mg/mL.

   **Note:** Freshly prepare the solution before each use.

8. The dosage is 60 mg/kg (Wu et al., 2013). Using a A769662 stock solution of 6 mg/mL, the volume of intraperitoneal injection into mouse = BW (g) × 10 μL/g.

## KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Chemicals, peptides, and recombinant proteins | | |
| MK8722 | MedChemExpress | Cat#HY-111363 |
| A769662 | Selleck | Cat#S2697 |
| Dimethyl sulfoxide (DMSO) | MP Biomedicals | Cat#196055 |
| PEG300 | Selleck | Cat#S6704 |
| Tween 80 | Selleck | Cat#S6702 |
| Saline solution (0.9% NaCl) | Sigma-Aldrich | Cat#S8776 |
| **Experimental models: organisms/strains** | | |
| Mouse: db/db mice (C57BL/KsJ) | Charles River | N/A |
| Mouse: db/db +/+ control mice (C57BL/KsJ) | Charles River | N/A |
| **Other** | | |
| Glucometer | Accu-Chek, Roche | N/A |
| Glucose test sticks | Accu-Chek, Roche | N/A |
| 1 mL LS 25GA 5/8 IN (0.5 mm × 16 mm RWLB) | Becton Dickinson Medical | 300841 |
| Dissection scissors | N/A | N/A |

**Note:** The diabetic db/db mice (C57BL/KsJ background), due to a mutation in the leptin receptor gene (Lept), develop type 2 diabetes spontaneously in adulthood and their AMPK signaling is significantly impaired. Therefore, male db/db mice at 12 weeks of age and their control +/+ mice are used to demonstrate the creditability and robustness of this protocol.

## STEP-BY-STEP METHOD DETAILS

### Fasting mice—day 1

© Timing: 30 min

1. Prepare 5–8 mice per group.

   **Note:** Metabolic assays in mice are diet-, age-, sex-, and strain-sensitive. Thus, it is important to keep these parameters comparable throughout each independent experiment.

2. Mice are housed with standard light–dark cycle and free access to water. They are fasted overnight for 12–16 h before test in clean cages with new bedding that has no food or feces.

   **Note:** For fasting, it is very important to remove all food and feces to avoid any pellet and stool intake.

### AMPK activator-induced glucose uptake—day 2

© Timing: 3 h

3. Start the procedure at 9 am.
**Note:** Metabolic studies should be performed at approximately the same hour during the day because physiological and biochemical parameters change throughout the day.

4. Prepare an experimental table for recording body weight (BW), the volumes of the stock solution of MK8722 or A769662 to be injected, and the glucose levels measured at different time points (t = 0, 15, 30, 45, 60, 90, and 120 min).

5. Weigh mice and record their BW in the experimental table.

**Note:** Handling mice gently to reduce their anxiety which may cause fluctuations in the blood glucose levels.

6. The dosing of MK8722 is 30 mg/kg, and A769662 is 60 mg/kg. Calculate and record in the table the volume of MK8722 or A769662 stock solution needed for intraperitoneal injection according to the BW of each mouse. The formula is as follows:

   Volume of MK8722 injection (µL) = BW (g) × 10 µL/g of 3 mg/mL MK8722 stock solution
   Volume of A769662 injection (µL) = BW (g) × 10 µL/g of 6 mg/mL A769662 stock solution.

7. Prepare syringes loaded with the required volume (according to step 6) of stock solution, glucometer, and glucometer sticks for measurements.

**Note:** Prepare one syringe per mouse with the corresponding volume of MK8722 or A769662 loaded according to its BW. Remove all air bubble from the syringes.

8. Cut 1–2 mm from the tail tip distal to the bone with a sharp dissection scissor (Figure 1).

9. Discard the first drop of blood.

![Figure 1. Cutting tail tips with a pair of sharp dissection scissors](image)
10. Let blood flow (2–5 μL) directly into the glucose test stick inserted into the glucometer and record the reading in the experimental table (t = 0).

Note: The amount of blood drawn from tail vein should be complied with the Institutional Guidelines for animal care and use. At Peking University, the maximum blood volume that may be drawn from a mouse per week is 6 μL/g of body weight.

11. Inject mice with syringes loaded with MK8722 or A769662.

Note: Space the injections of each mouse about 1 min apart to avoid overlapping of timings in blood glucose measurement. Make sure the injection is not subcutaneous by injecting at an angle below the visceral area. In obese mice, it is important to avoid fat pads when giving injections.

12. Start timer right after injection.

13. Repeat steps 10 at 15, 30, 45, 60, 90, and 120 min after MK8722 or A769662 injection.

14. Record the glucose values in the experimental table.

15. At the end of the experimental session, clean the mouse tails with sterile pads and place the mice in clean cages with free access to food and water.

16. Results are represented as percentage of basal blood glucose level overtime.

**EXPECTED OUTCOMES**

AMPK activator triggers glucose uptake in the control +/+ mice. In the diabetic db/db mice, the alteration in the blood glucose level induced by AMPK activator is significantly attenuated due to the impairment of AMPK signaling (Figure 2, Tables 1 and 2).

**QUANTIFICATION AND STATISTICAL ANALYSIS**

In Figure 2, results are presented as percentage of basal blood glucose level. Data are presented as mean ± SEM; *p<0.05, **p<0.005 versus corresponding controls. Raw data of blood glucose levels are shown in Table 1 (MK8722) and Table 2 (A769662). All p values below 0.05 were considered as significant (one-way ANOVA with the Bonferroni post-hoc test).

**LIMITATIONS**

It is recommended to carry out this test on no more than 15 mice each time. Measuring blood glucose takes time. Thus, handling too many mice at a time may reduce the accuracy of the timing.
for sampling, especially for the early time points. Injection of AMPK activators to the fasting +/+ control mice may cause seizures, even death, induced by hypoglycemia. However, fasting is necessary to make db/db mice sensitive to the blood glucose-lowering stimuli. The steps with “note” are the critical ones in the procedure.

TROUBLESHOOTING

Problem 1
Large variations in blood glucose levels.

Potential solution
This may be due to stress imposed on mice by the test. Handle the mice gently to reduce their anxiety.
Perform the experiments at the same hour of the day to avoid fluctuations in blood glucose levels. Check the expiration date of the glucose strips.

Problem 2
Blood glucose level exceeding the range of the glucometer.
Potential solution

Fasting db/db mice overnight to reduce their blood glucose levels to the measurable range. Simple dilution of blood from tail vein cannot give accurate estimation of the real blood glucose concentration, since the relationship between the readings and the blood glucose levels is not linear. We record the highest or lowest reading of the glucometer as the blood glucose level if the actual reading is out of range. This would not affect the final conclusion about AMPK activity.

Problem 3
Blood glucose level may be too low due to fasting which exceeds the range of glucometer.

Potential solution
In general, this should be avoided since severe hypoglycemia may be lethal to non-diabetic mice (+/+ control in this experiment). To prevent this situation, a dose titration of AMPK activator can be used to determine the best dosage for the test (see problem 5).

Problem 4
Need to study several groups of mice at the same time.

Potential solution
When there are multiple groups of mice to be examined and compared, the overall number of mice needed to be tested can easily exceeds 15. In this case, it is necessary to evenly divide mice into several small cohorts that contains similar number of mice from each and every group, and the total number of mice in each cohort is no more than 15. Run test for one small cohort each day, and incorporate all the data from all the cohorts for final analysis at the end.

Problem 5
Titration of dosage of AMPK activator.

Potential solution
It is recommended to perform a dose titration of stimuli before the real test. At least 3 dosages are recommended for titration. For MK8722, the dosages can be 15, 30, and 45 mg/kg. For A769662, the dosage can be 30, 60, and 90 mg/kg. Perform blood sampling and blood glucose measurement as shown in the protocol. The ideal dosage should be the one that gives a nice response curve in blood glucose level without causing sever hypoglycemia in normal mice.

RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Rui-Ping Xiao (xiaor@pku.edu.cn).

Materials availability
This study did not generate new unique reagents.

Data and code availability
This study did not generate new datasets or code.

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AUTHOR CONTRIBUTIONS

P.J., L.Z., and L.R. performed the experiments. L.Z., P.J., X.H., and R.-P.X. wrote the manuscript. All authors contributed to the manuscript and approved it for publication.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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