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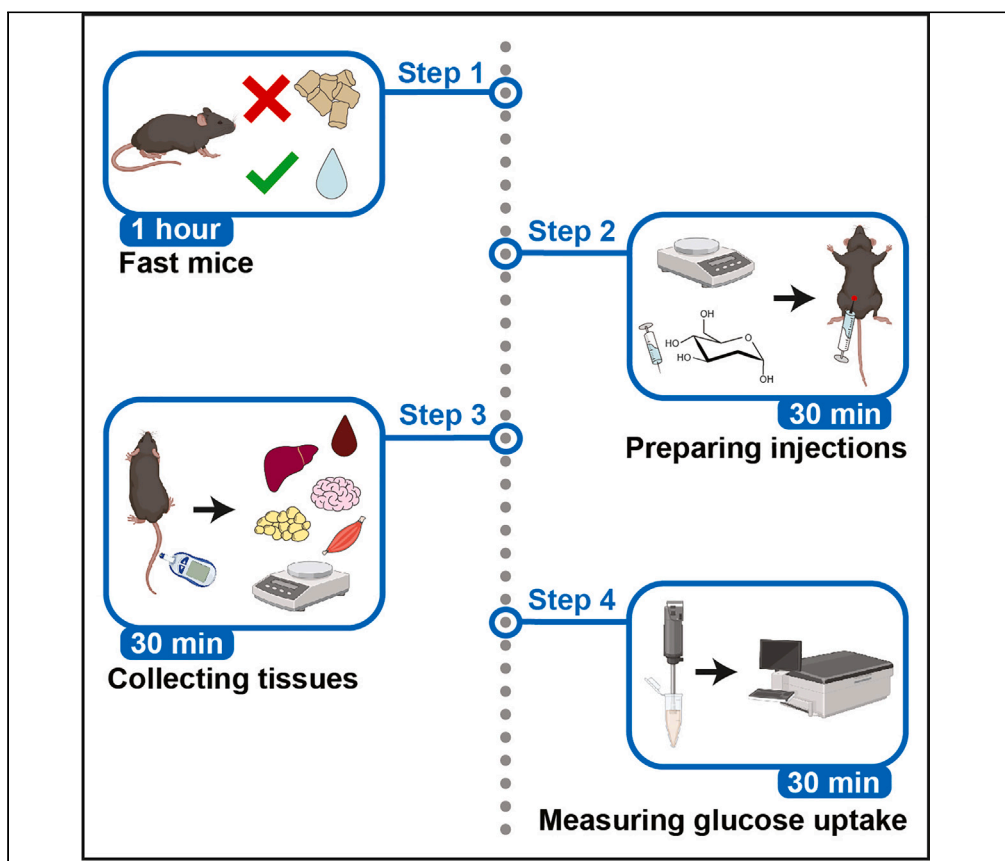
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Protocol

Protocol for *in vivo* measurement of basal and insulin-stimulated glucose uptake in mouse tissues

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Highlights

Protocol to quantitatively measure *in vivo* tissue-specific glucose uptake in mice

Steps for administration of radioactive glucose in the presence and absence of insulin

Description of tissue collection, sample processing, and data interpretation

Broadly applicable protocol for measuring tissue-specific glucose uptake

Here, we present an *in vivo* protocol for measuring basal and insulin-stimulated glucose uptake in tissues from mice. We describe steps for administering 2-deoxy-D-[1,2–³H]glucose in the presence or absence of insulin via intraperitoneal injections. We then detail tissue collection, tissue processing to measure ³H counts on a scintillation counter, and data interpretation. This protocol can be applied to other glucoregulatory hormones, genetic mouse models, and other species.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

Protocol

Protocol for *in vivo* measurement of basal and insulin-stimulated glucose uptake in mouse tissues

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SUMMARY

Here, we present an *in vivo* protocol for measuring basal and insulin-stimulated glucose uptake in tissues from mice. We describe steps for administering 2-deoxy-D-[1,2–³H]glucose in the presence or absence of insulin via intraperitoneal injections. We then detail tissue collection, tissue processing to measure ³H counts on a scintillation counter, and data interpretation. This protocol can be applied to other glucoregulatory hormones, genetic mouse models, and other species.

For complete details on the use and execution of this protocol, please refer to Jiang et al. (2021).[1]

BEFORE YOU BEGIN

In this protocol, glucose uptake is measured in four tissues and the serum: brown adipose tissue, quadriceps skeletal muscle, liver, and brain under basal and insulin-stimulated conditions using 2-deoxy-D-[1,2–³H]glucose. 2-deoxy-D-[1,2–³H]glucose has the 2-hydroxyl group replaced by hydrogen, which prevents its metabolism via glycolysis.[2] Unlike D-glucose, 2-deoxy-D-glucose accumulates intracellularly and therefore serves as a proxy of tissue-specific glucose uptake. Insulin induces glucose uptake in tissues expressing high levels of insulin-sensitive glucose transporters, such as quadriceps skeletal muscle and adipose tissue.[1,3,4]

Institutional permissions

Animal experiments in this study were performed per procedures approved by the Institutional Animal Care and Use Committee under the Stanford Animal Care and Use Committee (APLAC) protocol number #32982. All mice were in good health and housed in a temperature-controlled (20°C–22°C) room on a 12-h light/dark cycle with ad lib access to food and water. Before you begin, all animal experiments must be approved by the relevant institutions. Additionally, you must gain institutional approval for the use of radioactive materials in animals prior to purchasing and using the radioactive materials described here.

Preparation of insulin solution

© Timing: 10 min

1. Dissolve 12 mg insulin in 200 μL 0.1 N HCl.

⚠ CRITICAL: HCl can cause irritation or damage if inhaled or if it comes in contact with skin and eyes. HCl should be handled in the fumehood with appropriate PPE (i.e., lab coat, gloves, safety goggles). Insulin powder may be harmful if swallowed, inhaled, or in contact with skin. Appropriate PPE (i.e., lab coat, gloves) should be worn when handling.

2. Add 1,000 μL PBS to the insulin and HCl solution to generate an insulin stock concentration of 10 mg/mL or 270 U/mL.
3. Filter sterilize the insulin stock solution using a 0.22 μm filter, then aliquot and store in −80°C for up to 2 months.
4. To prepare the final insulin solution, dilute 2 μL insulin stock in 198 μL sterile saline to generate a final concentration of 2.7 U/mL.

   Note: The dose administered to mice will be 0.5–1 U/kg and should be calculated based on the body weight and administered in a final volume of 100–150 μL/mouse diluted in sterile saline.

Preparation of 2-deoxy-D-[1,2–^{3}H]glucose solution

   ⏱ Timing: 10 min

5. Prepare 2-deoxy-D-[1,2–^{3}H]glucose solution by mixing 100 μCi 2-deoxy-D-[1,2–^{3}H]glucose per kg body weight with saline to a total volume of 100 μL.
6. Prepare 2-deoxy-D-[1,2–^{3}H]glucose solution with insulin by adding the appropriate volume of 2-deoxy-D-[1,2–^{3}H]glucose to achieve a dose of 100 μCi/kg body weight and the appropriate volume of 2.7 U/mL insulin stock solution to achieve a dose of 0.5–1 U/kg body weight with saline to a total volume of 100 μL.

   Note: Prepare one tube containing the appropriate solution for each individual mouse.

   Note: The specific activity of 2-deoxy-D-[1,2–^{3}H]glucose is batch- and vendor-specific, so the exact volume of the stock solution has to be adjusted accordingly.

   ⚠ CRITICAL: Freshly prepare all solutions before each use. Do not freeze.

   ⚠ CRITICAL: Personal protective equipment (PPE) must be worn when handling radioactive materials. This includes a lab coat, safety goggles, and gloves. Radioactive experiments must be performed in designated and labeled areas. Radioactive sites should undergo site monitoring (i.e., swipe testing) monthly or weekly depending on institutional guidelines. Please refer to your institutional guidelines for institution-specific requirements for PPE and site monitoring.

   ⚠ CRITICAL: In the event that radioactive material is spilled, follow your institutional guidelines on how to handle spilled radioactive material. In general, don appropriate PPE, clean up the spill using absorbent materials, decontaminate the surface, and report the spill to the principal investigator. Survey the surrounding area and yourself using a radiation detector to ensure no further contamination.

   ⚠ CRITICAL: The use of radioactive 2-deoxy-D-[1,2–^{3}H]glucose requires institutional approval for proper handling and disposal.

   Alternatives: This protocol uses 2-deoxy-D-[1,2–^{3}H]glucose. However, other isotopes can be used, including 2-deoxy-D-[^{14}C]glucose. In general, 2-deoxy-D-[^{14}C]glucose is more stable

but for the short timeframe utilized in this method, the difference in stability will not affect the results.

Preparation of 1% SDS solution

⏲ Timing: 10 min

7. Dissolve 1 g SDS in 100 mL water to a final concentration of 1% (w/v).

⚠ CRITICAL: SDS powder is harmful if inhaled or swallowed and can cause skin irritation or serious eye damage. SDS powder should be handled in the fumehood with appropriate PPE (i.e., lab coat, gloves, safety goggles).

8. Keep solution at room temperature (20°C–22°C) until use. Discard unused solution.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Deoxy-D-glucose, 2-[1,2-^{3}H (N)]-, Aqueous Sol, Specific Activity: 5–10 Ci (185–370 GBq)/mmol	PerkinElmer	Cat#NET328A001MC
D-(+)-Glucose	Sigma-Aldrich	Cat# 50-99-7
Insulin, human recombinant	MilliporeSigma	Cat# 91077C
Saline solution (0.9% NaCl)	Teknova Inc	Cat# S5825
PBS	Corning	Cat#21-040-CM
HCl 0.1N	Sigma-Aldrich	Cat#2104
SDS	Sigma-Aldrich	Cat#436143
Experimental models: Organisms/strains		
Mus musculus: C57BL/6J (8-week-old males)	The Jackson Laboratory	Cat# 000664; RRID: IMSR_JAX:000664
Software and algorithms		
GraphPad Prism	GraphPad Prism	RRID: SCR_002798 https://www.graphpad.com/scientific-software/prism/
Other		
Glucometer	OneTouch UltraMini meter	N/A
Blood glucose strips	GenUltimate	Cat#100-50
Scintillation system	Beckman Coulter	LS6500
Ultima Gold Scintillation Fluid	Sigma-Aldrich	Cat#L8286
Gamma II vial, high density polypropylene, polyethylene snap-cap	Fisher Scientific	Cat#50-213-006
29G x 1/2 syringe	Comfort Point	Cat#26028
Fisherbrand 150 hand held homogenizer motor	Fisher Scientific	Cat#15-340-167
Fisherbrand 150 and 850 hand held homogenizer accessory, plastic disposable generator probe	Fisher Scientific	Cat#15-340-176
Millex-GS Syringe Filter Unit, 0.22 μm	Millipore Sigma	Cat#SLGSV255F
OHAUS Adventurer Analytical Balance	Fisher Scientific	Cat#01-920-251

MATERIALS AND EQUIPMENT

- **Solution A – 2-deoxy-D-[1,2-^{3}H]glucose solution:** For a 25 g mouse, add 2.5 μL of 2-deoxy-D-[1,2–^{3}H]glucose stock solution (1 μCi/μL) to 97.5 μL of sterile saline to create a 100 μL injection volume with a final dose of 100 μCi/kg.

Note: solution A should be made fresh for each experiment and never frozen.

Solution B – 2-deoxy-D-[1,2–³H]glucose solution with 1U/kg insulin (for a 25 g mouse):		
Reagent	Final concentration	Amount
2-deoxy-D-[1,2–³H]glucose (1 µCi/µL stock)	100 µCi/kg	2.5 µL
Insulin (2.7 U/mL stock)	0.5 U/kg	4.6 µL
Saline	N/A	92.9 µL
Total	N/A	100 µL

Note: solution B should be made fresh for each experiment and never frozen.

STEP-BY-STEP METHOD DETAILS
Fasting of mice

🕐 Timing: 1 h

This section details how to fast mice prior to glucose uptake assessment to reduce variability in blood glucose levels.

1. Prepare 5–10, 8-week-old male mice per experimental group.

   Note: All mice should be acclimatized for one week after import into the facility before the experiment.

   Note: Metabolic assays are dependent on the strain, age, sex, diet, and housing (single or group housed) conditions. Keep these parameters the same between experiments.

2. Transfer mice to new cages with clean, non-corn bedding with no food but allow free access to water.
3. Fast the mice for 1 h.

In vivo tissue-specific glucose uptake assay

🕐 Timing: 4 h

This step describes the administration of radioactive 2-deoxy-D-[1,2–³H]glucose into mice in the presence or absence of insulin, monitoring of blood glucose levels, tissue dissection, and processing of samples to measure tissue-specific glucose uptake.

4. Weigh mice and calculate the injection volume.
5. Administer **Solution A**: 2-deoxy-D-[1,2–³H]glucose, or **Solution B**: 2-deoxy-D-[1,2–³H]glucose with insulin by I.P. injection in a total volume of 100–150 µL/mouse.

   Note: When administering the 2-deoxy-D-[1,2–³H]glucose solutions, stagger the mice by 5–10 minutes to ensure all animals are sacrificed at the same time point after injection.

   Note: Metabolic studies should be performed at the same time of day due to circadian changes in physiological and biochemical parameters.

6. After 30 min, clean the tail with an ethanol wipe. Nick the tail vein with a razor blade. Use a glucometer to measure blood glucose.

⚠ CRITICAL: Do not omit the plasma blood glucose measurements as those serve as controls that insulin has been administered accurately. Typically, an insulin dose of 0.5–1 U/kg in lean, male C57BL/6J mice will reduce blood glucose levels by 50% from baseline after 15–30 minutes.

7. 30 min after the 2-deoxy-D-[1,2–³H]glucose injection, sacrifice animals and collect tissues in clean weighing boats.

⚠ CRITICAL: Always collect the tissues in the same order to avoid variability between animals and experiments.

8. Weigh and record the wet tissue mass using an analytical balance.

⏸ Pause point: The protocol can be paused after step 8 and tissues can be stored in −80°C for up to 2 weeks.

9. Transfer each tissue to a clean 2 mL snap cap tube.
10. Using a handheld homogenizer, homogenize each tissue in 300 μL 1% SDS for 1–2 min using a handheld homogenizer at room temperature (20°C–22°C).

Optional: Tissues can also be homogenized using a beadrupter. If so, replace snapcap tubes with O-ring tubes. Using radioactive materials in a beadruptor requires additional approvals. Consult institutional guidelines for radioactive materials before using a beadruptor.

11. Add the homogenized tissues to scintillation vials with 3.5 mL of Ultima Gold Scintillation Fluid. Cap the tubes and invert to mix.
12. Read samples on a scintillation counter (Beckman Coulter, LS6500) at 1 min per sample measuring ³H activity in counts per minute (CPM).

Optional: ³H activity can also be measured at 5 minutes per sample.

13. Calculate CPM/mg wet weight by dividing the CPM with the wet weight of each sample.
14. Depending on the need, measurements from tissues from the left and right side can be averaged upon data analysis.

EXPECTED OUTCOMES

These measurements provide information about changes in tissue-specific glucose levels under basal and insulin-stimulated states in male C57BL/6J mice (Figure 1A and Tables 1 and 2). Before injection, randomize mice to achieve non-significant differences in body weight between the saline and insulin treatment groups (Figure 1B). 30 min after insulin administration, plasma glucose levels will be reduced by 50% in the insulin group compared with the saline group (Figure 1C). These measurements serve as important positive controls that the mice have responded to the chosen dose of insulin. The expected relative glucose uptake across tissues is illustrated in Figure 1D, where brown adipose tissue (BAT) displays the highest absolute uptake of glucose per mg tissue weight, and also demonstrates the highest insulin-induced glucose uptake (Figure 1E). Quadriceps skeletal muscle demonstrates an intermediate level of glucose uptake, as well as a significantly induced insulin-stimulated glucose uptake (Figure 1F). Liver and brain demonstrate intermediate levels of glucose uptake, but expectedly, no insulin-induced glucose response (Figures 1G and 1H). Given that the liver lacks the insulin-responsive glucose transporter GLUT4, it is expected that insulin treatment would not alter glucose uptake in the liver in lean mice.[5] However, glucose uptake in the liver may be altered in diabetic or obese animals, thus it is important to measure glucose uptake in the liver. Similarly, the major glucose transporter in the brain is GLUT3, which is also not responsive to insulin, thus
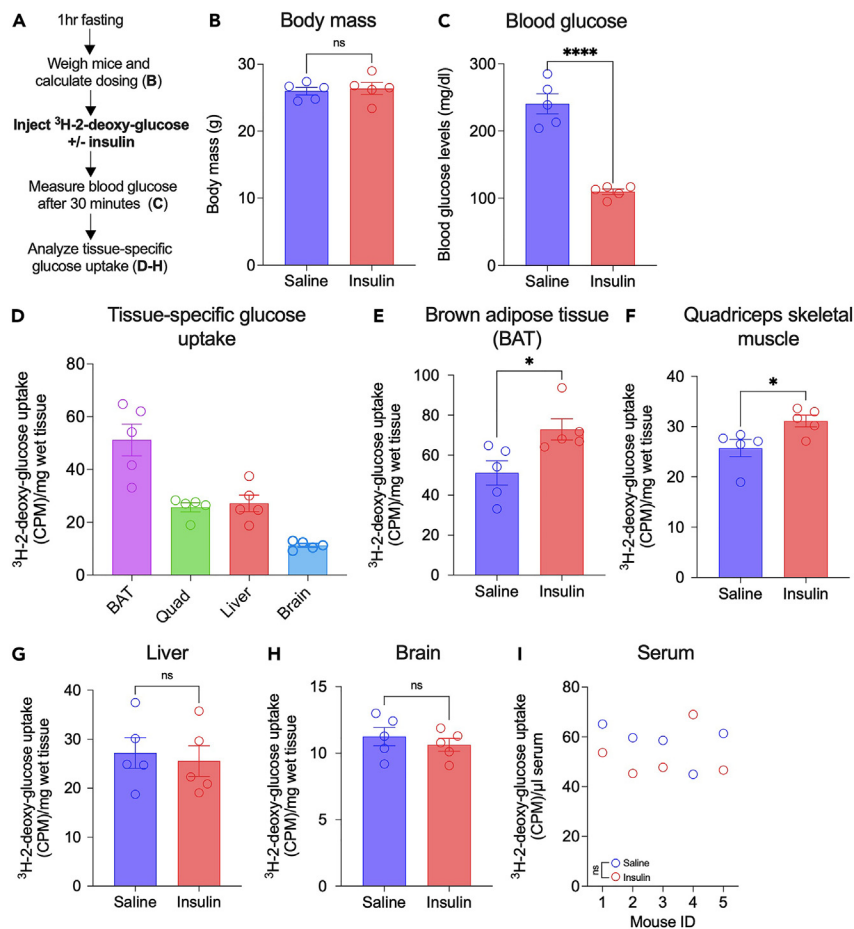
Figure 1. Basal and insulin-stimulated tissue-specific glucose uptake in male C57BL/6J mice

(A) Timeline and main steps of the *in vivo* glucose uptake assay.

(B) Typical body mass of 8-week-old wild-type, male C57BL/6J mice fed a chow diet (n = 5 animals/group).

(C) Blood glucose levels (mg/dl) 30 min after I.P. injection of vehicle or 0.7 U/kg insulin (n = 5 animals/group).

(D) Comparison of basal glucose uptake levels after 30 min across brown adipose tissue (BAT), quadriceps skeletal muscle (quad), liver, and brain expressed as 2-deoxy-D-[1,2–^{3}H]glucose (CPM) per mg of wet tissue weight (n = 5 animals/group).

(E) Glucose uptake after 30 min in brown adipose tissue (BAT) under basal and insulin-stimulated conditions expressed as 2-deoxy-D-[1,2–^{3}H]glucose (CPM) per mg of wet tissue weight (n = 5 animals/group).

(F) Glucose uptake after 30 min in quadriceps skeletal muscle under basal and insulin-stimulated conditions expressed as 2-deoxy-D-[1,2–^{3}H]glucose (CPM) per mg of wet tissue weight (n = 5 animals/group).

(G) Glucose uptake after 30 min in the liver under basal and insulin-stimulated conditions expressed as 2-deoxy-D-[1,2–^{3}H]glucose (CPM) per mg of wet tissue weight (n = 5 animals/group).

(H) Glucose uptake after 30 min in the brain under basal and insulin-stimulated conditions expressed as 2-deoxy-D-[1,2–^{3}H]glucose (CPM) per mg of wet tissue weight (n = 5 animals/group).

(I) Glucose uptake after 30 min in the serum under basal and insulin-stimulated conditions expressed as 2-deoxy-D-[1,2–^{3}H]glucose (CPM) per μL of serum (n = 5 animals/group).

All data are presented as mean ± SEM; *$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ versus corresponding controls calculated using Student's *t*-test.

we do not expect insulin to alter glucose uptake in the brain.[6] Additionally, serum radioactivity levels can also be measured as a control to demonstrate that the 2-deoxy-D-[1,2–^{3}H]glucose injections were successful and not different between mice in different conditions (Figure 1I). This protocol can be used under other physiological conditions e.g., treatment with other glucoregulatory hormones, genetic mouse models, diet conditions, or other rodent or non-rodent species.

Table 1. Raw data of tissue-specific glucose uptake 30 min after vehicle or insulin injection presented as counts per minute (CPM)

Tissue type	Brown fat (left)	Brown fat (right)	Quadriceps muscle (left)	Quadriceps muscle (right)	Liver (piece 1)	Liver (piece 2)	Brain	Serum
Control (no treatment)	4	2	3	11	5	14	12	4
A1. 2-deoxy-D-[1,2–^{3}H]glucose + saline	1424	1728	3805	3966	2219	2224	4333	652
A2. 2-deoxy-D-[1,2–^{3}H]glucose + saline	1527	1512	4347	4743	1997	2121	5094	597
A3. 2-deoxy-D-[1,2–^{3}H]glucose + saline	1605	1800	5227	5638	2335	2277	4922	586
A4. 2-deoxy-D-[1,2–^{3}H]glucose + saline	1404	1616	4075	4361	2246	2261	3916	450
A5. 2-deoxy-D-[1,2–^{3}H]glucose + saline	1852	1615	2279	2829	2129	2177	4349	614
B1. 2-deoxy-D-[1,2–^{3}H]glucose + insulin	2352	2127	4171	4468	1915	2067	4455	537
B2. 2-deoxy-D-[1,2–^{3}H]glucose + insulin	2922	2265	4591	5271	2357	2446	4343	453
B3. 2-deoxy-D-[1,2–^{3}H]glucose + insulin	2409	2210	4906	5243	1692	1730	4094	478
B4. 2-deoxy-D-[1,2–^{3}H]glucose + insulin	2746	3045	5814	5954	1628	1675	4776	690
B5. 2-deoxy-D-[1,2–^{3}H]glucose + insulin	2120	2238	4338	4587	2237	2275	4649	467

Optional: Instead of normalizing tissue radioactivity to wet tissue mass, you can also normalize tissue radioactivity to the dose of 2-deoxy-D-[1,2–^{3}H]glucose. Both options will facilitate glucose uptake comparisons between tissues and between mice.

QUANTIFICATION AND STATISTICAL ANALYSIS

Student's *t*-test is sufficient to compare two groups with a p-value < 0.05 considered statistically significant. If comparing multiple groups, use ANOVA for multiple comparisons. GraphPad Prism is used for the statistical analyses of the results.

LIMITATIONS

This protocol is optimal to evaluate glucose uptake in tissues within a relatively short-term time frame (15 min up to 1 h). The accuracy of this method for glucose uptake measurements at timepoints longer than 1 h has not been evaluated. Another limitation is the sample processing time. It is recommended to limit the number of animals in each experiment to no more than 20 at a time.

TROUBLESHOOTING

Problem 1
Large biological variations in glucose uptake between animals.

Potential solution

- For comparative studies, only use animals from the same source, such as littermate controls or age-matched animals purchased from the same vendor at the same time (step 1).
- Ensure that the animals are housed in non-corn bedding during the fasting period and that no food is hidden in the bedding (step 2).
- Ensure that all animals are sacrificed at the exact same time point after 2-deoxy-D-[1,2–^{3}H]glucose administration by staggering the injection and tissue harvesting times (step 5).

Problem 2
Large technical variations in glucose uptake.

Potential solution

- Administer 2-deoxy-D-[1,2–^{3}H]glucose in a sufficiently large volume. No less than a total volume of 100 μL per animal is recommended to reduce variability between animals (step 5).
- Reduce the number of animals handled in a single experiment to less than 20 (steps 1 and 5).

Table 2. Raw data of organ masses (g) and serum volume (μL)

Tissue type	Brown fat (left)	Brown fat (right)	Quadriceps muscle (left)	Quadriceps muscle (right)	Liver (piece 1)	Liver (piece 2)	Brain	Serum
Control (no treatment)	0.0389	0.0424	0.154	0.1382	0.077	0.082	0.4086	10
A1. 2-deoxy-D-[1,2–³H]glucose + saline	0.0286	0.0295	0.132	0.1495	0.063	0.0889	0.4182	10
A2. 2-deoxy-D-[1,2–³H]glucose + saline	0.0515	0.0412	0.1442	0.2077	0.085	0.0817	0.3919	10
A3. 2-deoxy-D-[1,2–³H]glucose + saline	0.022	0.0352	0.1867	0.196	0.0573	0.0666	0.3962	10
A4. 2-deoxy-D-[1,2–³H]glucose + saline	0.028	0.0488	0.1643	0.1485	0.1101	0.077	0.426	10
A5. 2-deoxy-D-[1,2–³H]glucose + saline	0.0312	0.023	0.13	0.1386	0.1212	0.109	0.3855	10
B1. 2-deoxy-D-[1,2–³H]glucose + insulin	0.0294	0.0198	0.1462	0.1195	0.0773	0.1042	0.4131	10
B2. 2-deoxy-D-[1,2–³H]glucose + insulin	0.038	0.0398	0.1638	0.1627	0.128	0.1047	0.4295	10
B3. 2-deoxy-D-[1,2–³H]glucose + insulin	0.03	0.035	0.1857	0.1888	0.0577	0.0577	0.4514	10
B4. 2-deoxy-D-[1,2–³H]glucose + insulin	0.042	0.043	0.1837	0.1672	0.071	0.1101	0.402	10
B5. 2-deoxy-D-[1,2–³H]glucose + insulin	0.03	0.0389	0.1273	0.1569	0.0717	0.0564	0.4115	10

- Record the wet tissue weight by weighing each tissue immediately after dissection (steps 7 and 8).
- Ensure that the entire tissue has been fully homogenized before transferring the homogenate to scintillation vials (step 10).

Problem 3
No observed insulin-induced glucose uptake in insulin-responsive tissues.

Potential solution

- Prepare fresh insulin stock solution in saline the day of the experiment (step 4).
- Increase the insulin dose (step 5) and/or increase the fasting period (step 3) before insulin injection. Titrate the dose of insulin or perform an insulin tolerance test in the animals that will be tested because of large variability in the needed insulin doses.
- The plasma blood glucose measurements will ensure that insulin has been administered accurately (step 6).

Problem 4
Working with diabetic or insulin resistant mice.

Potential solution

- Some animal strains (ob/ob, db/db and others) are highly insulin-resistant and may need a higher insulin dose or a longer fasting period before the insulin injection (step 5).
- Working with insulin resistant animals may also require an extended time between injection and tissue harvesting (steps 6 and 7).
- Insulin sensitivity in tissues may vary in insulin resistant animals compared to lean animals. Thus, it is advisable to include a control group of lean, 8-week-old wild-type mice which are typically highly insulin sensitive to ensure that the administered insulin is active (step 1).

Problem 5
Large variations in serum radioactivity levels.

Potential solution

- Large variations in serum radioactivity levels may suggest technical errors in the preparation or administration of injections (step 4).
- Ensure that the concentration of 2-deoxy-D-[1,2–³H]glucose and insulin were calculated correctly based on the body weight of each mouse (step 4).

- Ensure that injections are administered in the correct location and to the correct depth (step 5).
- Small injection volumes can have difficulty dispersing homogenously. Thus, the injection volume can be increased to facilitate equal dispersion of 2-deoxy-D-[1,2–^{3}H]glucose throughout the body (step 4).

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Katrin J. Svensson (katrinjs@stanford.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

This study did not generate new datasets or code.

AUTHOR CONTRIBUTIONS

M.Z. performed the experiments. M.Z., L.W.W., and K.J.S. wrote the manuscript. All authors contributed to the manuscript and approved it for publication.

DECLARATION OF INTERESTS

K.J.S. is a member of the Advisory Board at STAR Protocols.

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