

Insulin Tolerance Test and Hyperinsulinemic-Euglycemic Clamp

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[Abstract] The two tests are used to evaluate *in vivo* sensitivity to insulin in mouse. The hyperinsulinemic-euglycemic clamp provides information about the sensitivity to insulin in liver and other metabolically relevant tissues.

Materials and Reagents

1. Human insulin (Eli Lilly, Indianapolis, IN)
2. [3-³H] glucose (Perkin Elmer, catalog number: NET331A250UC)
3. 2-deoxy-D-[1-¹⁴C] glucose (2-[¹⁴C]DG) (PerkinElmer, catalog number: NET328250UC)

Equipment

1. Contour blood glucometer (Bayer)

Procedure

1. C57BL/6J mice were fasted for 6 h and then injected intraperitoneally with insulin (1 U per kg of body weight), and blood glucose concentrations were monitored over time using a Contour blood glucometer on a drop of blood from the tip of the tail.
2. Mice were cannulated in the lateral cerebral ventricle and catheterized in the right internal jugular vein for the hyperinsulinemic-euglycemic clamp (Figure 1) (Thrivikraman *et al.*, 2002). Intravenous infusion of [3-³H] glucose (5 µCi bolus, 0.05 µCi/min) was used.
3. Human insulin (16 mU/kg) was injected intravenously as a bolus, followed by continuous infusion at 2.5 mU/kg/min.
4. Tail blood glucose was measured by glucometer at 10 min intervals, and 20% glucose was infused to maintain blood glucose at euglycemic levels (120 to 140 mg/dl of plasma glucose levels).
5. After steady state had been maintained for 1 h, the glucose uptake in various tissues was determined by injecting 2-deoxy-D-[1-¹⁴C] glucose (2-[¹⁴C]DG) (10 mCi) 45 min before the end of clamps (the catheter was used for the injection). During the final 50 min of basal

and clamp infusions, 20 μ l blood samples were collected at 10 min intervals for measurement of [3 H] glucose, [3 H] H₂O and 2-[14 C]DG from the tail vein. Samples were stored in -20 °C.

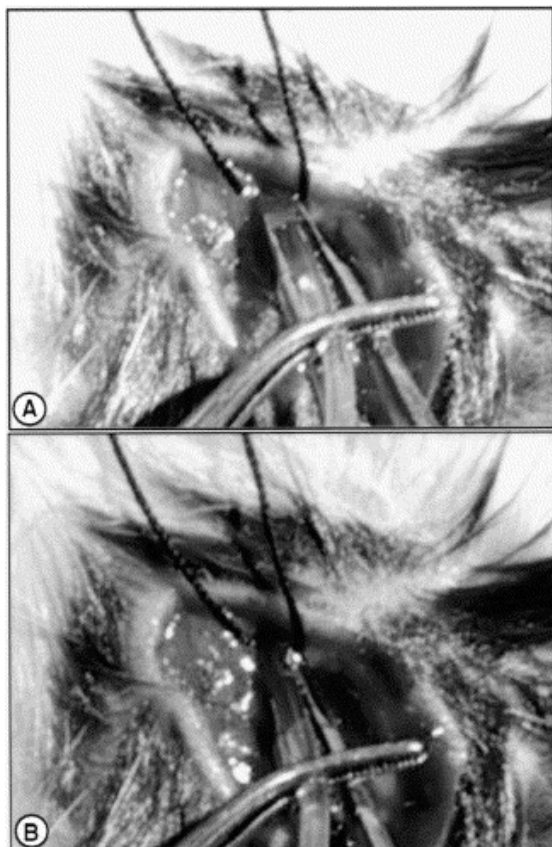


Figure 1. Right internal jugular vein catheterization. A catheter is placed in the right jugular vein for the infusion of glucose and insulin.

Acknowledgments

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References

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