Dose-Response Relationship of Insulin to Glucose Fluxes in the Awake and Unrestrained Mouse

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The purpose of the study was to use the hyperinsulinemic-euglycemic clamp technique to generate insulin dose-response curves for insulin suppression of endogenous glucose output (EGO) and stimulation of the glucose disposal rate (GDR) in conscious unstressed mice. Five groups of male ICR (Institute for Cancer Research) mice were studied (N = 43). The animals underwent surgery for implantation of a jugular vein catheter 2 to 3 days before the clamp and were fasted 6 hours before the study. Each group was clamped at a different insulin infusion rate of 0, 2.5, 10, or 20 mU/kg/min. ^{3}H-3-glucose was infused for measurement of the glucose turnover rate (rate of appearance [Ra]). Blood samples were collected by milking a severed tail-tip. EGO was calculated as the difference between the Ra and glucose infusion rate (GIR), and the glucose clearance rate (GCR) as the GDR divided by the plasma glucose concentration. From the curves generated, half-maximal EGO and GCR were obtained at a plasma insulin concentration of 20 to 30 μU/mL, which was achieved at an insulin infusion rate of about 4 to 5 mU/kg/min. Maximal suppression of EGO and stimulation of the GCR occurred at an insulin infusion rate of 10 mU/kg/min. The establishment of normative curves for insulin-stimulated glucose metabolism in conscious mice facilitates the evaluation of glucose metabolism in a variety of mouse models of insulin resistance.

IN VIVO STUDIES of glucose metabolism have been critical for the evaluation of insulin sensitivity. Insulin resistance is common to many pathophysiological states, including obesity, non–insulin-dependent diabetes mellitus, and hypertension. One of the most useful techniques to quantify in vivo insulin action is the combination of a hyperinsulinemic glucose clamp and tracer methodology[1-7] to assess the rates of insulin-stimulated glucose disposal and endogenous glucose output (EGO).[1,2]

This technique has been applied in humans,[5,7-9] dogs,[10-13] goats,[14] rabbits,[15] and rats.[16-19] Because of technical difficulties, the clamp technique has been applied only infrequently in the mouse. This is unfortunate, because the large number of transgenic and knockout mouse models produced in recent years[20-23] makes it desirable that a reliable technique for in vivo evaluation of the molecular physiology of glucose metabolism be available. Only recently has the glucose clamp technique been used to assess insulin sensitivity in mice. In 1994, Marshall and Mueckler[24] used glucose infusion rates (GIRs) required to achieve steady-state glucose concentrations during hyperinsulinemic glucose clamps to assess insulin sensitivity in anesthetized mice. The disadvantage of using anesthetized animals is that the anesthetic itself causes significant alterations of physiological function and actually causes insulin resistance, thus underestimating true insulin action and potentially confounding interpretation of the data.[25,26] Since 1995, a few laboratories have conducted glucose clamp studies combined with tracer infusion in awake mice.[27,28] However, these studies have not revealed the full insulin dose-effect on glucose disposal and suppression of EGO in mice. Such normative data are important to better evaluate insulin action in various mouse models of insulin resistance.

Herein, we describe in detail the hyperinsulinemic-euglycemic clamp technique in combination with tracer glucose infusion applied in the awake and unrestrained mouse to reveal the effect of the full insulin dose-response range on whole-body glucose disposal and EGO in non-obese, nonhypertensive, and nondiabetic mice. The curves obtained from these studies may serve as a normative reference for other studies using the clamp technique in various mouse models of insulin resistance.

MATERIALS AND METHODS

Animals

Male ICR mice (Institute for Cancer Research, descended from Charles River Laboratories, Wilmington, MA, and Harlan, Indianapolis, IN) weighing 37.15 ± 0.39 g were used (Table 1). Animals were housed in the Indiana University Laboratory Animal Research Center on a 12-hour light/dark cycle with standard chow and water available ad libitum. The study was approved by the Animal Use Committee at Indiana University School of Medicine.

Mice were anesthetized for cannulation of the jugular vein. Ketaset 1 mL/kg (mixture of ketamine, acepromazine, and atropine; Fort Dodge Laboratory, Fort Dodge, IA) was injected into the peritoneal cavity, and the right jugular vein was exposed. Catheters were prepared as follows: PE-10 polyethylene tubing (ID 0.28 mm, OD 0.61 mm; Becton Dickinson, Parsippany, NJ) almost 7 mm long was attached to a piece of PE-50 (ID 0.58 mm, OD 0.61 mm; Becton Dickinson) with Medical Adhesive Silicone Type A (Dow Corning, Midland, MI). A small silastic-tubing ring 2 mm in length (ID 0.064 mm, OD 0.12 mm; Dow Corning) was set out of the PE-10 and PE-50 connection to form a node to help immobilize the catheter with ligatures. The ready-to-use catheter was then connected to a syringe via a 0.65-mm blunted needle (Terumo Medical, Elkton, MD) with isotonic saline (0.9%). The PE-10 tubing filled with saline was inserted into the right atrium through the right jugular vein and was anchored with silk ligatures. Catheters were tunneled under the skin and exteriorized in the nuchal area. The catheters were filled with isotonic saline to prevent reflux of blood into the lumen and then capped with ballpoint pins (blunt tips). Since mice are potentially vulnerable to such surgery (approximately 25% mortality rate), they were kept under warming lights until they gained

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Table 1. Characteristics of the Study Animals

Characteristic	Insulin Dose (mU/kg/min)				
	0	2.5	5	10	20
No. of mice	8	12	8	7	8
Weight (g)	37.0 ± 1.1	37.2 ± 0.5	39.0 ± 0.7	38.1 ± 0.9	38.8 ± 1.1
Fasting glucose (mg/dL)	92.3 ± 9.7	91.3 ± 8.1	98.1 ± 10.6	87.6 ± 9.3	95.4 ± 8.1
Fasting insulin (μU/mL)		7.0 ± 2.1 (n = 20)			
Basal EGO (mg/kg/min)		38.6 ± 1.8 (n = 11)			

NOTE. Values are the mean ± SE.

consciousness and began to move. Subsequently, animals were placed into the cage for usual care. For animals that appeared weak (hypokinetic), infusions of saline and 20% dextrose solution (Abbott Laboratories, North Chicago, IL) were performed with the aim of increasing the survival.

Hyperinsulinemic Glucose Clamp Studies

Two to 3 days of rest were allowed for the animals to recover from surgery. Food was withheld overnight, and euglycemic clamp studies were performed while the animals were awake, unrestrained, and unstressed in their regular cages. Evidence that the animals were physiologically ready for study included healthy physical appearance, normal activity, and weight regained after surgery.

Five groups of mice were studied, each at a different insulin infusion rate of 0, 2.5, 5, 10, or 20 mU/kg/min, respectively (Table 1). Insulin (Humulin-R; Eli Lilly & Co, Indianapolis, IN) was dissolved in 0.9% saline to form infusates. After obtaining the basal plasma glucose concentration, a venous catheter was connected to infusion syringes and insulin infusion was initiated at time 0 with a compact Harvard pump (model 975; Harvard Apparatus, South Natick, MA) at a rate of 0.15 mL/h. The blood glucose concentration was measured at 10-minute intervals and clamped at euglycemia with a variable infusion of 20% dextrose using an adjustable-rate pump (model A-99; Razel Scientific Instruments, Stanford, CT). The tail tip was severed, and blood samples were collected for glucose measurement by milking the tail tip. Blood glucose concentrations were determined by the glucose oxidase method with a glucometer (Accu-Check Advantage; Boehringer Mannheim, Indianapolis, IN). Clamp GIRs were calculated from the mean of the GIRs obtained during the last 30 minutes of the clamp. Plasma insulin concentrations were measured by a double-antibody radioimmunoassay (Linco, St Louis, MO) using a rat insulin antibody to measure fasting insulin levels and a human insulin antibody to measure levels during the clamp studies. Clamp insulin levels were obtained when GIRs achieved steady state, approximately 60 to 100 minutes after the start of the insulin infusion. A total of 46 studies were performed in an equal number of mice.

Determination of Glucose Turnover

The glucose rate of appearance (Ra) in both the basal and insulin-stimulated states was determined isotopically. ^{3}H-3-glucose 0.65 μCi (New England Nuclear, Boston, MA) in 21.5 μL saline (0.9%), 5 μL of which is for the dead space of the tubing, was first injected as a bolus dose followed by a constant infusion of ^{3}H-3-glucose 0.075 μCi/min (3 μL/min). Blood samples (100 μL)[19] were obtained for measurement of ^{3}H-3-glucose specific activity at the end of each clamp (approximately 60 to 100 minutes), by which time plasma glucose was stabilized at basal levels. Duplicates were made by transferring plasma samples into two tubes with 50 μL in each. They were then deproteinized with 60% perchloric acid (Fisher Scientific, Fair Lawn, NJ). Perchloric acid 100 μL/50 μL plasma was mixed and centrifuged for 20 minutes. The supernatant (75 μL) was then evaporated, resuspended in 75 μL deionized water followed by the addition of 3 mL liquid scintillation fluid (Ecoscint; National Diagnostics, Manville, NJ), and counted (Liquid Scintillation Analyzer model A2100; Packard, Downers Grove, IL).

The recovery of counts was determined by mixing a known volume of plasma from control mice with an aliquot of tracer solution of known dpm and analyzed in the counting procedure together with the experimental plasma samples. A recovery rate of about 100% was usual, and thus, it was not determined in all assays.

The basal Ra was calculated by dividing the rate of infusion of ^{3}H-3-glucose in dpm (dpm per minute = infusate dpm specific for a given mouse × infusate infusion rate) by the plasma glucose specific activity (dpm per milligram) obtained at the end of the 60-minute ^{3}H-3-glucose infusion. The Ra in mice undergoing insulin stimulation was measured by dividing the infusion rate in dpm by the plasma glucose specific activity sampled at the end of each hyperinsulinemic glucose clamp.

EGO

EGO represents the residual EGO from hepatic and renal sources during insulin infusion. The difference between the Ra and GIR represents EGO (EGO = Ra − GIR). In cases in which the Ra was underestimated (ie, less than the GIR), EGO was considered to be zero.

Glucose Disposal Rate and Clearance Rate

When the Ra was greater than the GIR, the glucose disposal rate (GDR) was considered equal to the Ra. When the Ra was less than the GIR, the GIR was considered representative of the GDR.

Since the clamped glucose concentrations were slightly different from animal to animal, the glucose utilization rate between animals and between groups may not be directly comparable by using the GDR alone. Therefore, we also calculated the glucose clearance rate (GCR) to represent the GDR adjusted for the prevailing steady-state glucose concentrations: GCR (milliliters per kilogram per minute) = GDR (milligrams per kilogram per minute)/plasma glucose (milligrams per milliliter).

Data Analysis

Data are reported as the mean ± SEM. Comparisons between groups were performed with ANOVA using the Statview 4.0 program (Abacus Concepts, Berkeley, CA) followed by a Fisher protected least-significant difference test. A P level less than .05 was considered statistically significant.

RESULTS

Characteristics of the Mouse Groups

The body weight of the animals used in this study was not different between groups (P = .94). After jugular vein cannulation, the animals usually lost 2 to 3 g body weight. The mean weight presurgery was 40.1 ± 0.5 g and decreased to 38.2 ± 0.5 g before the clamp study. The mean fasting plasma glucose

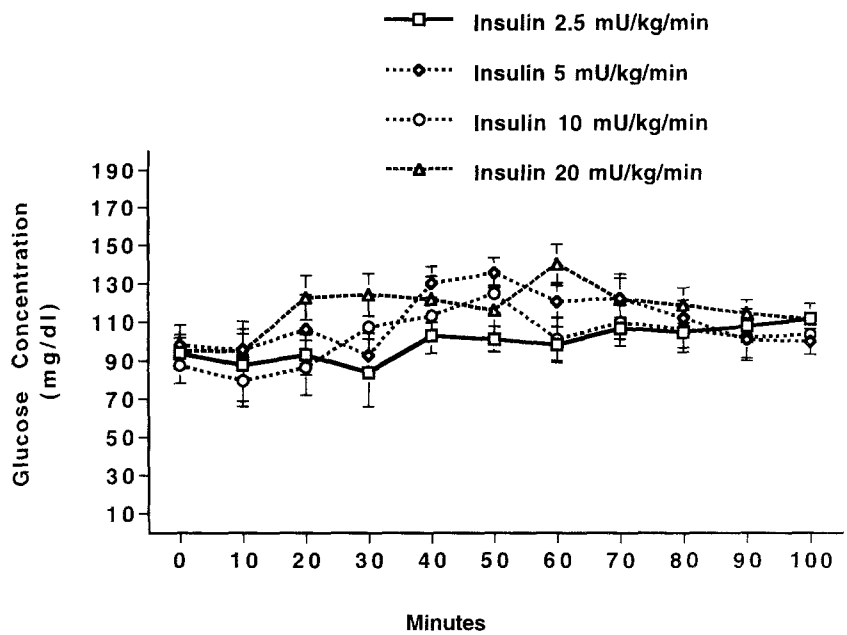

Fig 1. Plasma glucose concentration at baseline and during hyperinsulinemic-euglycemic clamp in 4 groups of mice studied at different insulin infusion rates.

concentration in these mice was 94.9 ± 4.0 mg/dL. The fasting insulin level in a subgroup of 20 mice was 7.0 ± 2.1 μU/mL. In 35 clamp studies, steady-state euglycemia was achieved within 60 to 100 minutes with a mean plasma glucose concentration of 111.3 ± 3.0 mg/dL (Table 1).

Hyperinsulinemic-Euglycemic Clamp Studies

Figure 1 illustrates plasma glucose concentrations during the clamp. Plasma glucose was usually stable by 70 to 80 minutes after the start of the clamp, and the GIR was virtually constant by 60 minutes. The time course of the GIR at different insulin concentrations is shown in Fig 2, which illustrates a graded increase in glucose uptake in response to the insulin dose ($P < .001$, ANOVA). At both physiologic and pharmacologic insulin concentrations, the onset of insulin action was rapid and the increase of glucose uptake could be detected within 30 minutes after the start of insulin infusion (Fig 1).

Two ways of collecting blood samples for measurement of clamp insulin concentrations were applied and compared. One way was to sample the blood from the tail tip freshly cut 5 minutes before the end of the clamp. Another way was to collect blood following mice decapitation at the end of the study. Steady-state plasma insulin concentrations (clamp insulin) at insulin infusion rates of 2.5, 5, 10, and 20 mU/kg/min were 4.2 ± 1.1, 21.2 ± 5.7, 104.1 ± 27.9, and 122.1 ± 44.9 μU/mL, respectively, from tail tip blood. When blood was collected by

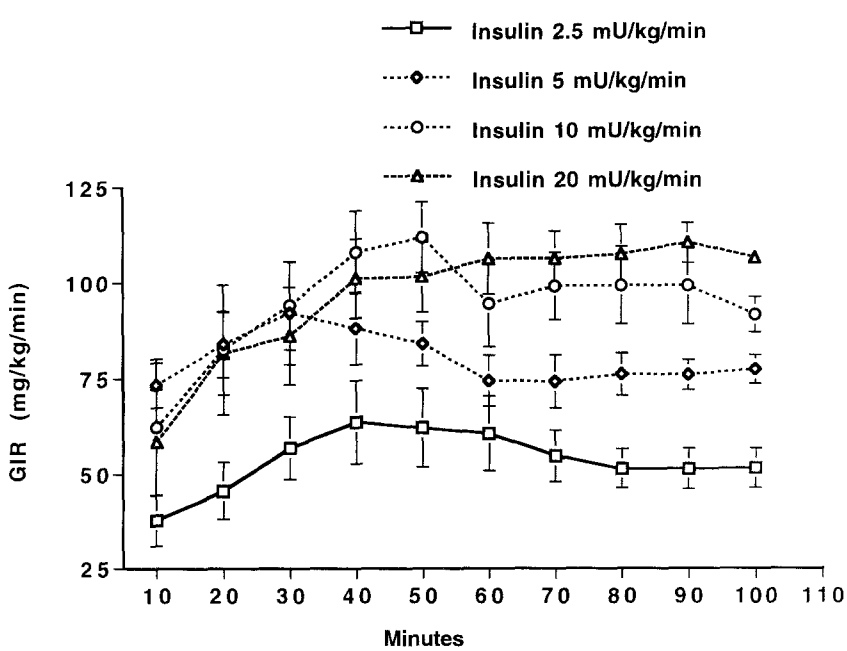

Fig 2. GIR during hyperinsulinemic-euglycemic clamp in 4 groups of mice studied at different insulin infusion rates.

decapitation, the clamp insulin concentrations were 71.7 ± 20.3, 91.4 ± 39.5, 436.7 ± 153.6, and 459.9 ± 154 μU/mL, respectively. The latter results were used for Fig 3.

To examine the dose-response effects of insulin on glucose turnover and EGO, the GDR and residual EGO were measured at the various insulin concentrations. At insulin doses of 2.5, 5, and 10 mU/kg/mL, the GDR was 77.3 ± 10.2, 85.2 ± 3.5, and 102.7 ± 7.9 mg/kg/min, respectively. The GDR was maximal (118.3 ± 5.4 mg/kg/min) at the highest insulin dose of 20 mU/kg/min. Basal EGO in the fasting state was 38.6 ± 1.5 mg/kg/min. At insulin infusion rates of 2.5, 5, 10, and 20 mU/kg/min, EGO was suppressed to 28.2 ± 3.8, 10.6 ± 3.0, 0.0 ± 0.0, and 0.0 ± 0.0 mg/kg/min, respectively. This indicates complete suppression of EGO at an insulin infusion rate of 10 mU/kg/min (Fig 3).

The GCR[29] at insulin infusion rates of 2.5, 5, 10, and 20 mU/kg/min was 0.486 ± 0.061, 0.775 ± 0.062, 1.023 ± 0.109, and 1.117 ± 0.067 dL/kg/min, respectively (Fig 3).

The dose-response relationship between the plasma insulin concentration and the whole-body GCR and EGO is illustrated in Fig 3. A half-maximal inhibition of EGO and stimulation of the GCR can be estimated from this curve to occur at an insulin infusion rate of approximately 4.0 mU/kg/min.

DISCUSSION

This study was designed to construct the full dose-response curve for insulin's action to suppress EGO and stimulate whole-body glucose disposal in conscious unstressed mice. The results of the clamp studies demonstrate a dose-dependent effect of insulin to stimulate glucose uptake in mice. The sigmoidal-shaped dose-response curves are similar to those obtained in humans and rats.[19,30] At maximally effective insulin concentrations, the rate of insulin-mediated glucose uptake was about four times greater than the basal rate. This fold-stimulation is somewhat less than that in rats and humans, which appear to be fivefold and sixfold the basal rate, respectively.[30] At lower insulin doses (0 to 2.5 mU/kg/min), glucose clearance increased modestly; however, at higher insulin doses, the increase in glucose uptake was steep and almost linear, and appeared to reach a maximal rate at an approximate insulin infusion rate of 10 mU/kg/min. Glucose uptake did not increase as insulin infusion was increased from 10 to 20 mU/kg/min.

Based on the curves generated in Fig 3, the insulin infusion rate required to achieve half-maximal stimulation of the GCR and half-maximal suppression of EGO could be estimated at about 4 to 5 mU/kg/min. The maximally effective insulin infusion rate for stimulation of the GCR and suppression of EGO can also be estimated from the curve at approximately 10 mU/kg/min.

We compared two ways of collecting blood samples for measurement of basal and clamp insulin concentrations. The first was to sample the blood from the tail tip freshly cut 5 minutes before the end of the clamp. Another way was to collect blood following decapitation of the mice at the end of the study. It appears that insulin measurements obtained from neck blood were generally higher than those obtained from tail-cutting (*P* values for 0, 2.5, 5, 10, and 20 mU/kg/min insulin infusion were .001, .02, .07, .05, and .057). The reason for these concentration differences is not immediately clear, but they may have resulted from the dilution of tail blood by tissue fluid. Therefore, the actual insulin concentrations at which half-maximal and maximal biologic effects occur are not possible to determine with certainty, but are greater than 20 and 100 μU/mL, respectively.

Blood sampling is a major hurdle to overcome in clamping mice. Because of the limitations of small body weight and blood volume, it was not possible to draw blood from the venous cannula for measurement of glucose concentrations, as is the case for rats. We found it more practical to milk the tail to obtain blood samples sufficient for glucose measurements. Usually, playing with the mice for a few minutes prior to study helped them to become accustomed to handling, which presumably minimized any stress during the blood milking.

In this study, we calculated three different parameters to gauge insulin's effect to stimulate glucose uptake. The GIR is a valid measure of the GDR when EGO is completely suppressed under the conditions of sufficient hyperinsulinemia. In our study, this situation was achieved at an insulin infusion rate of 10 mU/kg/min, achieving insulin levels between approximately 100 and 400 μU/mL. When insulinemia is not sufficient to completely suppress EGO, the GIR itself underestimates the

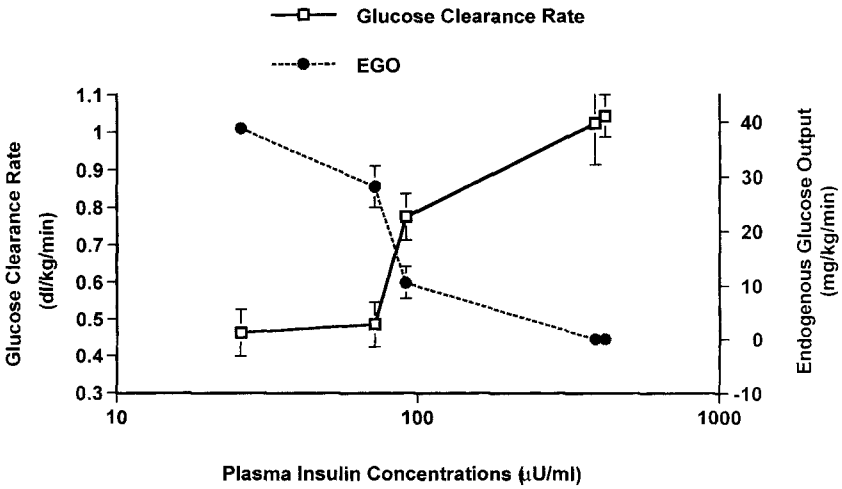

Fig 3. Dose-response relationship between plasma insulin concentration and insulin-mediated GCR and residual EGO in awake unrestrained mice.

true rate of glucose uptake. Under steady-state conditions, when EGO is not completely suppressed, the Ra is greater than the GIR, and the actual GDR should be derived from the Ra. A comparison of rates of insulin-stimulated glucose turnover achieved at similar insulin concentrations is only valid insofar as the plasma glucose is clamped at the same level. An approach to dealing with modestly different clamp plasma glucose levels is the calculation of the GCR,[29] ie, GDR/plasma glucose concentration. The GCR is the effect of insulin to enhance glucose utilization independently of differences in the prevailing glucose concentration.[29,31] The GCR is only valid within a narrow range of glucose concentrations, because the effect of the glucose mass to enhance glucose uptake is not linear beyond the tissue K_m (affinity constant) for glucose uptake. Because steady-state glucose concentrations differed slightly between clamp studies (<10%), we calculated the GCR to compare the insulin effect on glucose uptake between animals.

In summary, we have described the technique to perform glucose clamp studies in conscious mice and established the full dose-response relationship between the insulin concentration and insulin action in healthy mice. The clamp procedure in mice is difficult but feasible and relatively successful when carefully conducted. This technique can be extremely useful to quantify in vivo insulin sensitivity in various genetically engineered mice models currently produced to study insulin signaling and action. The dose-response curve reported here can serve as normative data to better determine the specific insulin dose for studies to assess insulin sensitivity and responsiveness in various mouse models of insulin resistance and diabetes.

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REFERENCES

1 Molina JM, Baron AD, Edelman SV, et al: Use of a variable tracer infusion method to determine glucose turnover in humans. Am J Physiol 258:E16-E23, 1990

2. Hother-Nielsen O, Henriksen JE, Holst JJBN: Effects of insulin on glucose turnover rates in vivo: Isotope dilution versus constant specific activity technique. Metabolism 45:82-91, 1996

3. Steele R, Wall JS, deBodo RC, et al: Measurement of size and turnover rate of body glucose pool by the isotope dilution method. Am J Physiol 187:15-24, 1956

4. Steele R: Influences of glucose loading and injected insulin on hepatic glucose output. Ann NY Acad Sci 82:420-430, 1959

5. Andres R, Swerdloff R, Pozefsky T, et al: Manual feedback technique for the control of blood glucose concentration. in Skeggs LT Jr (ed): Automation in Analytical Chemistry. New York, NY, Mediad, 1966. pp 486

6. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: A method for quantifying insulin secretion and resistance. Am J Physiol 237:E214-E223, 1979

7. Sherwin RS, Kramer KJ, Tobin JD, et al: A model of the kinetics of insulin in man. J Clin Invest 53:1481-1492, 1974

8. Baron AD, Wallace P, Brechtel G: In vivo regulation of non–insulin mediated and insulin mediated glucose uptake by cortisol. Diabetes 36:1230-1237, 1987

9. Baron AD, Brechtel G, Wallace P, et al: Rates and tissue sites of non–insulin and insulin mediated glucose uptake in humans. Am J Physiol 255:E769-E774, 1988

10. Rebrin K, Steil GM, Getty L, et al: Free fatty acid as a link in the regulation of hepatic glucose output by peripheral insulin. Diabetes 44:1038-1045, 1995

11. Miles PD, Levisetti M, Reichart D, et al: Kinetics of insulin action in vivo. Identification of rate-limiting steps. Diabetes 44:947-953, 1995

12. Christopher MJ, Rantzau C, Ward GM, et al: Impact of variable insulinemia and glycemia on in vivo glycolysis and glucose storage in dogs. Am J Physiol 266:E62-E71, 1994

13. Fisher SJ, Shi ZQ, Lickley HL, et al: A moderate decline in specific activity does not lead to an underestimation of hepatic glucose production during a glucose clamp. Metabolism 45:587-593, 1996

14. Tesseraud S, Grizard J, Makarski B, et al: Effect of insulin in conjunction with glucose, amino acids and potassium on net metabolism of glucose and amino acids in the goat mammary gland. J Dairy Res 59:135-149, 1992

15. Mossberg KA, Taegtmeyer H: Time course of skeletal muscle glucose uptake during euglycemic hyperinsulinemia in the anesthetized rabbit: A fluorine-18-2-deoxy-2-fluoro-D-glucose study. J Nucl Med 33:1523-1529, 1992

16. Kraegen EW, James DE, Bennett SP, et al: In vivo insulin sensitivity in the rat determined by euglycemic clamp. Am J Physiol 245:E1-E7, 1983

17. Proietto J, Rohner-Jeanrenaud F, Ionescu E, et al: Non–steady-state measurement of glucose turnover in rats by using a one-compartment model. Am J Physiol 252:E77-E84, 1987

18. Rossetti L, Smith D, Shulman GI, et al: Correction of hyperglycemia with phlorizin normalizes tissue sensitivity to insulin in diabetic rats. J Clin Invest 79.1510-1515, 1987

19. Smith D, Rossetti L, Ferrannini E, et al: In vivo glucose metabolism in the awake rat: Tracer and insulin clamp studies. Metabolism 36·1167-1174, 1987

20. Manchester J, Skurat AV, Roach P, et al: Increased glycogen accumulation in transgenic mice overexpressing glycogen synthase in skeletal muscle. Proc Natl Acad Sci USA 93:10707-10711, 1996

21. Marshall BA, Ren JM, Johnson DW, et al: Germline manipulation of glucose homeostasis via alteration of glucose transporter levels in skeletal muscle. J Biol Chem 268:18442-18445, 1993

22. Ren JM, Marshall BA, Gulve EA, et al: Evidence from transgenic mice that glucose transport is rate-limiting for glycogen deposition and glycolysis in skeletal muscle. J Biol Chem 268:16113-16115, 1993

23. Gulve EA, Ren JM, Marshall BA, et al: Glucose transport activity in skeletal muscles from transgenic mice overexpressing GLUT1. Increased basal transport is associated with a defective response to diverse stimuli that activate GLUT4. J Biol Chem 269:18366-18370, 1994

24. Marshall BA, Mueckler MM: Differential effects of GLUT-1 or GLUT-4 overexpression on insulin responsiveness in transgenic mice. Am J Physiol 267:E738-E744, 1994

25. Penicaud L, Ferre P, Kande J, et al: Effect of anesthesia on glucose production and utilization in rats. Am J Physiol 252:E365-E369, 1987

26. Rossetti L, Klein-Robbenhaar G, Giebisch G, et al: Effect of insulin on renal potassium metabolism. Am J Physiol 252:F60-F64, 1987

27. Ren JM, Marshall BA, Mueckler MM, et al: Overexpression of Glut4 protein in muscle increases basal and insulin-stimulated whole body glucose disposal in conscious mice. J Clin Invest 95:429-432, 1995

28. Hebert LF Jr, Daniels MC, Zhou J, et al: Overexpression of glutamine:fructose-6-phosphate amidotransferase in transgenic mice leads to insulin resistance. J Clin Invest 98:930-936, 1996

29. Bergman RN, Finegood DT, Ader M: Assessment of insulin sensitivity in vivo. Endocr Rev 6:45-86, 1985

30. Smith D, DeFronzo RA: Insulin resistance in uremia mediated by postbinding defects. Kidney Int 22:54-62, 1982

31. Reaven GM, Bernstein R, Davis B, et al: Nonketotic diabetes mellitus: Insulin deficiency or insulin resistance? Am J Med 60:80-88, 1976