Intravenous Glucose Tolerance Test–Derived Glucose Effectiveness in Endurance-Trained Rats

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Glucose tolerance is determined by complex interaction among several physiological processes, including insulin secretion, insulin sensitivity (SI), and the action of glucose itself to enhance glucose uptake and suppress its release by the liver. The combined effect of glucose to enhance glucose uptake and suppress endogenous glucose production at basal insulin has been defined as glucose effectiveness (SG) and is at least equal to insulin itself in the determination of glucose tolerance. The present study was undertaken to examine the effects of endurance training on SG in young rats. Female Sprague-Dawley rats (7 weeks old) were randomly assigned to sedentary and trained groups, and rats in the trained group had access to a running wheel for 3 weeks. Intravenous glucose tolerance tests ([IVGTTs] 500 mg/kg body weight) were performed 30 hours after the rats stopped wheel-running. The glucose disappearance constant (KG) and minimal model–derived SI and SG for trained rats were higher than for sedentary animals. Increases in SG were positively correlated with the relative heart weight, an index of endurance capacity. Our results confirm the previous observation in a cross-sectional study that an improved glucose tolerance in endurance-trained athletes was due to an increase in SG and SI, suggesting that physical exercise is a unique physiological condition that enhances both SG and SI.

GLUCOSE TOLERANCE is determined by complex interaction among several physiological processes, including the insulin secretory response to a glucose load, the sensitivity of insulin-responsive tissue to the action of secreted insulin, and the action of glucose itself to enhance glucose uptake and suppress its release by the liver. The combined effect of glucose to enhance glucose uptake and suppress endogenous glucose production at basal insulin has been defined as glucose effectiveness (SG).[1] Although its exact physiological meaning and cellular basis remain to be determined, SG is at least equal to insulin itself in the determination of glucose tolerance.[1] Physical exercise is unique since it is the only known physiological condition to enhance SG and insulin sensitivity (SI). A single bout of exercise enhances SG and SI in untrained individuals.[2,3] We have also shown in a cross-sectional study that improved glucose tolerance in endurance-trained atheletes was due to an increase in SG and SI.[4] Endurance training has the potential to enhance SG, although longitudinal studies in middle-aged subjects did not detect a statistically significant effect.[5,6] In the present study, we evaluated this issue by studying the effects of endurance training on SG in young rats.

MATERIALS AND METHODS

Animals and Research Design

Female Sprague-Dawley rats (6 weeks old) were obtained from CLEA Japan (Tokyo, Japan). All procedures involving the animals were approved by the ethics committee of the University of Tsukuba. The rats were housed at 23°C with light from 7 AM to 7 PM and with free access to water and powdered chow (CE-2; CLEA Japan) except that the food was removed from the cages 6 hours before the intravenous glucose tolerance test (IVGTT). Rats aged 7 weeks were weight-matched and separated into sedentary and training groups. Rats in the sedentary group were kept in individual wire-bottomed cages (15 × 25 × 17 cm), and rats in the training group were kept in individual wire-bottomed cages (15 × 25 × 15 cm) with a running wheel (circumference, 1 m; KC-8000; Tokushima Medical, Tokushima, Japan) to which the rats had free access for 3 weeks as described previously.[7,8]

Thirty hours before the IVGTT (ie, by 7 AM), the animals underwent placement of a tail artery blood sampling catheter and tail vein infusion catheter (PE10; Clay Adams, Parsippany, NJ). Catheters were placed percutaneously during local anesthesia with lidocaine (1% wt/vol) while the animals were briefly restrained. After catheter placement, the animals were returned to their cages with tail restraints.[9] The catheters were maintained by infusion (0.6 mL/h using a digital infusion pump; Harvard Apparatus, Boston, MA) of heparinized (2 U/mL) physiological saline.

IVGTT

Protocol I: IVGTT. IVGTTs were performed at 1 PM. Food was removed from the cages at 7 AM on the morning of the IVGTT (6-hour fasted). All animals received a bolus injection of glucose (500 mg/kg body weight as a 20% solution in water) into a tail vein. Tail arterial samples (100 μL) were taken into syringes containing heparin (0.1 U) and NaF (1 mg) at −5, 2, 4, 6, 8, 10, 12, 14, 16, 19, 22, 25, 30, 40, and 60 minutes. Plasma was frozen at −30°C for subsequent analysis.

Protocol II: IVGTT with suppressed dynamic insulin response. The basal sample was drawn at −30 minutes. To suppress the endogenous insulin response to a glucose load, somatostatin was infused systemically through a tail vein (3 μg/kg/min)[10] beginning at −25 minutes and continuing throughout the experiment, which lasted until 80 minutes. After a bolus injection of glucose (500 mg/kg body weight) into a tail vein, tail arterial samples were taken at −5, 2, 5, 10, 15, 20, 30, 40, 60, and 80 minutes.

Assays

Plasma glucose levels were measured in duplicate spectrophotometrically with glucose oxidase (Glucose B-test; Wako Pure Chemical Industries, Osaka, Japan). The immunoreactive insulin content in 5 μL of plasma was determined in duplicate using the ELISA Insulin kit (Seikagaku, Tokyo, Japan) with rat insulin as a standard.

Data Analysis

SI and SG were estimated by the minimal model approach.[11-14] The minimal model program was written in Pascal (Borland International, Scotts Valley, CA) on a Macintosh computer (Apple Computer,

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Cupertino, CA). Briefly, the Marquardt-Lebenberg method was used for nonlinear least-square estimation of the parameters, and values at 0 to 4 minutes were zero-weighted. The step size for the integration was 0.2 minutes.

KG was calculated as the slope of the least-square regression line relating the natural logarithm of the glucose concentration to time between 5 and 15 minutes. The endogenous plasma insulin response was expressed as the area of the plasma insulin curve during the first 10 minutes above basal as described previously.[15]

Statistics

The data are presented as the mean ± SE. To evaluate differences between two means, the data were analyzed by Student's *t* test. Differences were considered statistically significant at *P* less than .05.

RESULTS

Running-Wheel Activity, Food Intake, Body Weight, and Tissue Weight

Running-wheel activity increased rapidly in the first 10 days to a plateau and food intake increased after a transient decrease in the training group (Fig 1). The training group gained less weight than the sedentary group. The weight of abdominal adipose tissue and gastrocnemius muscle was significantly less and the heart weight was significantly greater in the training group than in the sedentary group (Table 1).

IVGTT

Plasma glucose and insulin concentrations during the IVGTT are shown in Fig 2a and b. There was no statistically significant difference in basal glucose (120 ± 2 *v* 124 ± 3 mg/dL, sedentary *v* training) and basal insulin (86 ± 10 *v* 90 ± 8 pmol/L). After the intravenous glucose load, plasma glucose increased equally in both groups. Like in other species, including humans,[3,4] plasma glucose decreased to less than basal before returning to the basal level. The glucose disappearance constant (KG) as an estimate of glucose tolerance was significantly higher in trained than in sedentary rats. After glucose administration, trained rats tended to have lower plasma insulin than the sedentary group, but the difference was not statistically significant. The integrated area of plasma insulin during the first 10 minutes was similar between the groups. SI, SG, and glucose effectiveness at zero insulin (GEZI) were higher in trained rats than in sedentary animals, but the difference in the basal insulin effect (BIE) was not statistically significant (Table 2).

IVGTT With Suppressed Dynamic Insulin Response

Somatostatin infusion reduced basal insulin (from 79.4 ± 10.6 to 7.2 ± 4.5 pmol/L in the sedentary group and from 97.3 ± 36.1 to 16.8 ± 9.6 pmol/L in the training group), and the dynamic insulin response was suppressed by 70% in both groups. Compared with the IVGTT in protocol I, KG values were significantly reduced in the IVGTT with suppressed dynamic insulin response in both groups. KG values were significantly higher in trained versus sedentary rats (Table 2).

DISCUSSION

Glucose disappearance rates vary considerably among different animal species. It takes about 90 to 120 minutes for dogs and humans[3,4,11,15] but only 60 minutes for rats to reestablish the basal glucose level. It is therefore reasonable to assume that an IVGTT can be performed within 60 minutes in rats. KG values in dogs, monkeys, and rabbits are about 3.5, 6.0 to 8.0, and 1.5% · min^{-1}, respectively.[16-20] In the normal human, KG is about 2.0% · min^{-1},[21] and it can be as high as 3.60 ± 0.64% · min^{-1} in endurance-trained athletes[4] and lower than 1.0% · min^{-1} in glucose-intolerant subjects and diabetic pa-

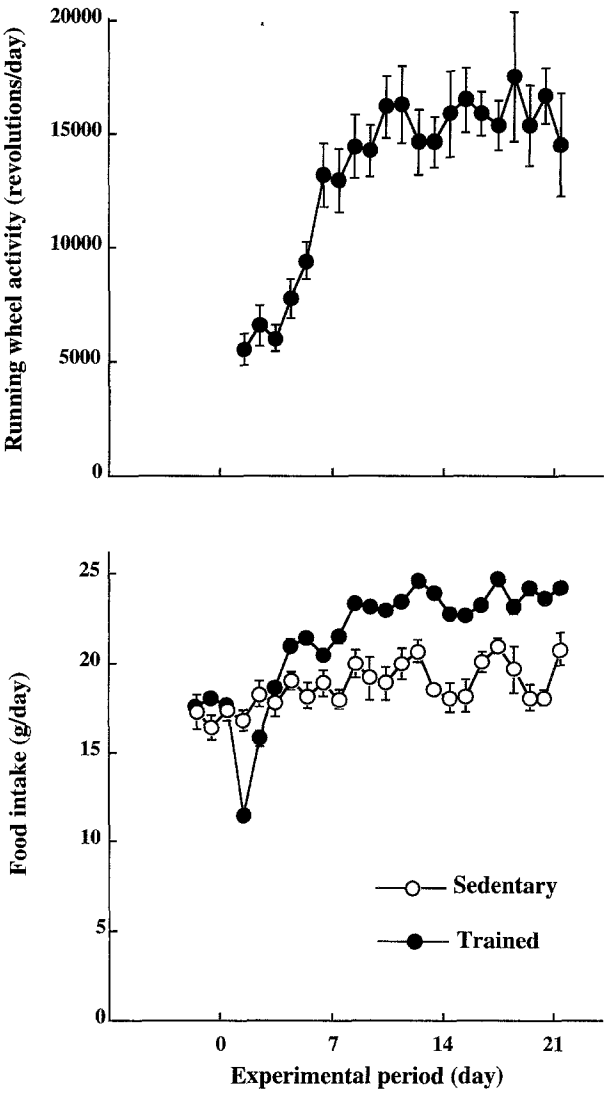

Fig 1. Changes in running-wheel activity and food intake. Values are the mean ± SEM. Wheel running began at age 7 weeks (day 1 of the experimental period).

Table 1. Tissue Weight (g) in Sedentary and Trained Rats

Parameter	Sedentary	Trained
Initial body weight	170 ± 4	170 ± 3
Final body weight	230 ± 3	218 ± 4*
Abdominal adipose tissue	3.33 ± 0.29	1.53 ± 0.19†
Soleus muscle	0.20 ± 0.01	0.21 ± 0.01
Gastrocnemius muscle	2.57 ± 0.05	2.28 ± 0.05†
Heart	0.72 ± 0.01	0.76 ± 0.01*

*$P < .05$, †$P < .01$: *v* sedentary.

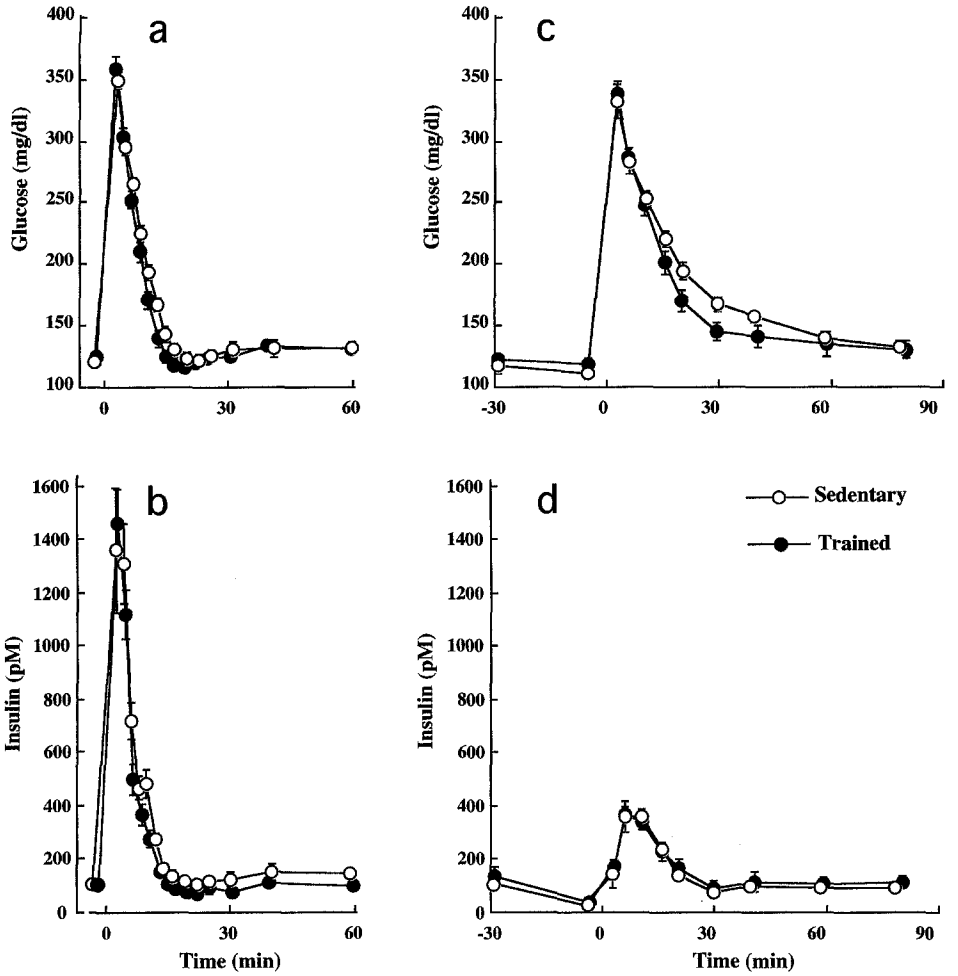

Fig 2. Time course of plasma glucose and insulin concentrations during the IVGTT (a and b) and the IVGTT with suppressed dynamic insulin (c and d). Values are the mean ± SEM.

tients.[15,22] The KG value of 7.0% · min^{-1} for sedentary rats in the present study is comparable to the previously reported value[23] and higher than for most other species already mentioned. Endurance training further improved glucose disappearance in the rats, confirming previous reports.[24]

Trained rats had a higher SI and SG than sedentary animals, consistent with our previous study on endurance-trained athletes.[4] Application of the minimal model approach to experimental animals was initially limited to dogs, with which the minimal model was originally developed,[11] but it has recently been applied to monkeys[20] and pigs.[25] The original protocol of minimal model analysis requires 33 samples for blood glucose and insulin measures during the IVGTT.[11] Recent development of a reduced-sample protocol for the IVGTT[26-28] and microassay of plasma insulin (5 μL) allowed us to perform an IVGTT with rats. For humans, but not for dogs, tolbutamide or insulin injection is required to improve the accuracy of parameter estimation by minimal model analysis.[29,30] It has not been determined whether the same treatment improves the accuracy of minimal model analysis in a rat IVGTT. Due to technical difficulties such as a rapid change in plasma glucose in rats and an unavailability of rat insulin, etc., we chose to perform the IVGTT without tolbutamide or insulin injection, sampling as frequently as possible with the current sampling technique. A larger dose of glucose (500 mg/kg) was chosen to induce adequate insulin secretion. Our animal study confirmed previous findings in humans, such as an enhanced SI and SG in endurance-trained athletes[4] and a negative correlation of SI to adiposity.[21] Although it remains to be proven that the values for SG and SI are valid, it is still reasonable to assume that the physiological basis of the minimal model, ie, "remote insulin" stimulates peripheral glucose disposal and inhibits net hepatic glucose output and glucose per se enhances net glucose disposal independently of the insulin response, can be generally applied to the rat, the dog, and the human. In the present study, IVGTTs

Table 2. IVGTT Parameters in Sedentary and Trained Rats

Parameter	Sedentary	Trained
Protocol I: IVGTT	n = 10	n = 10
KG (% · min^{-1})	7.00 ± 0.45	9.24 ± 0.78*
SI (10^4 · min^{-1} · $pmol/L^{-1}$)	1.21 ± 0.16	2.05 ± 0.37*
SG (min^{-1})	0.091 ± 0.011	0.147 ± 0.017*
GEZI (min^{-1})	0.081 ± 0.011	0.129 ± 0.013*
BIE (min^{-1})	0.010 ± 0.001	0.018 ± 0.005
Insulin area (pmol/L · min)	6,362 ± 1,136	5,854 ± 607
Protocol II: IVGTT with suppressed dynamic insulin response	n = 7	n = 7
KG (% · min^{-1})	2.64 ± 0.27	3.84 ± 0.39*‡
Insulin area (pmol/L · min)	1,838 ± 536	1,836 ± 165‡

*$P < .05$, †$P < .01$: *v* sedentary.
‡$P < .01$ *v* protocol I.

with somatostatin infusion were also performed to directly estimate SG. Since the dynamic insulin response was not completely suppressed, SG could not be estimated from this experiment. Glucose tolerance in trained rats was still better than in sedentary rats when the dynamic insulin response to a glucose load was greatly but not completely suppressed. Taken together, these results suggest that endurance training by 3 weeks of wheel running enhances glucose effectiveness in rats.

Endurance-trained athletes had higher SI and SG than sedentary subjects when tested 16 hours and 1 week after the last training session. The effect of a single bout of exercise on non–insulin-mediated glucose uptake (NIMGU) can last for more than several hours after exercise ends,[31-34] but is reversed within 18 hours in rats fed carbohydrate.[31,35] Since the IVGTT was performed 30 hours (24 hours of feeding and 6 hours of fasting) after tail artery cannulation, it is likely that the enhanced SI and SG observed in trained rats were due to a long-term effect of endurance training rather than to the residual effect of the last bout of exercise greater than 30 hours before the IVGTT. In either case, the present longitudinal study shows that rats trained with a running wheel have higher SI and SG in the habitual state. SG consists of two components: GEZI and BIE. GEZI, which is more closely related to NIMGU than SG itself, is the major component of SG (88% ± 2% of SG in the sedentary group and 89% ± 2% of SG in the trained group) in rats. GEZI was higher in the trained group than in the sedentary group.

Consistent with a previous human study,[21] SI was negatively correlated with abdominal adipose tissue weight ($R^2 = .423$, $P < .01$), suggesting the possibility that enhanced SI in trained rats is related to reduced adiposity. On the other hand, SG is not correlated with adiposity or skeletal muscle mass, but is positively correlated with the relative heart weight ($R^2 = .319$, $P < .05$), suggesting that better-trained animals acquired higher SG. GEZI was also positively correlated with relative heart size ($R^2 = .322$, $P < .05$). However, in a population-based study, SG was not significantly associated with maximal oxygen consumption in 186 males (mean ± SD, 44 ± 9 mL/min/kg) and 194 females (38 ± 8 mL/min/kg).[21] In our previous cross-sectional study comparing sedentary subjects and endurance-trained athletes, the difference in maximal oxygen consumption was 37% (40.9 ± 1.4 *v* 56.2 ± 1.2 mL/min/kg) and endurance-trained athletes had higher SG.[4] To our knowledge, two longitudinal studies have been conducted with middle-aged subjects to assess the effect of physical training on SG. Kahn et al[5] compared SG in healthy older men (68.6 years) before and after 6 months of endurance training and found no effect of exercise training on SG (0.014 ± 0.001 *v* 0.015 ± 0.002 min^{-1}). Although their exercise training produced an endurance training effect, the increase in maximal oxygen consumption was only 18%. Similarly, Houmard et al[6] found no significant increase in SG (0.020 ± 0.002 *v* 0.023 ± 0.002 min^{-1}) after 14 weeks of training that increased maximal oxygen consumption by 20%. Therefore, it seems that only intensive endurance training increases SG (Fig 3). Clarification of whether SG is related to physical fitness, particularly the endurance-trained state, deserves further attention.

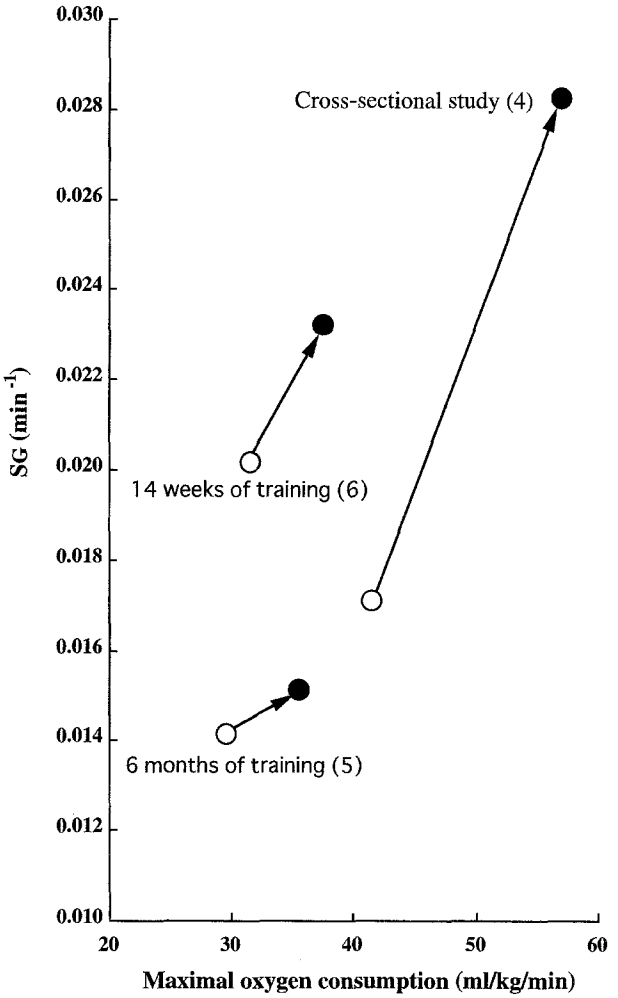

Fig 3. Relation between maximal oxygen consumption and SG in human studies. Maximal oxygen consumption was significantly higher in the trained group (●) *v* the sedentary group (○) in all studies. The difference in SG was statistically significant in a cross-sectional study,[4] but not in longitudinal studies in middle-aged subjects.[5,6]

SG is at least equal to insulin itself in the determination of glucose tolerance in humans.[1] Its quantitative importance inevitably led to recent efforts to identify factors that determine SG. It has been suggested that the mechanism by which glucose acts to increase cellular metabolism independently of a change in insulin involves mass action through GLUTs already located on the cell surface, transporter recruitment,[36] and enzyme activation.[37-39] It remains to be determined endurance training enhances SG, and further physiological study combined with biochemical analysis of tissues is clearly warranted.

REFERENCES

1. Best JD, Kahn SE, Ader M, et al: Role of glucose effectiveness in the determination of glucose tolerance. Diabetes Care 19:1018-1030, 1996

2. Brun JF, Guintrand-Hugret R, Bouix O, et al: Influence of short-term submaximal exercise on parameters of glucose assimilation analyzed with the minimal model. Metabolism 44:833-840, 1995

3. Higaki Y, Kagawa T, Fujitani J, et al: Effects of a single bout of exercise on glucose effectiveness. J Appl Physiol 80:754-759, 1996

4. Tokuyama K, Higaki Y, Fujitani J, et al: Intravenous glucose tolerance test–derived glucose effectiveness in physically trained humans. Am J Physiol 265:E298-E303, 1993

5. Kahn SE, Larson VG, Beard JC, et al: Effect of exercise on insulin action, glucose tolerance, and insulin secretion in aging. Am J Physiol 258:E937-E943, 1990

6. Houmard JA, Shinebarger MC, Dolan PL, et al: Exercise training increases GLUT-4 protein concentration in previously sedentary middle-aged men. Am J Physiol 264:E896-E901, 1993

7. Tokuyama K, Saito M, Okuda H: Effects of wheel running on food intake and weight gain of male and female rats. Physiol Behav 28:899-903, 1982

8. Tokuyama K, Okuda H: Fatty acid synthesis in adipose tissue of physically trained rats in vivo. Am J Physiol 245:E8-E13, 1983

9. Buchanan TA, Youn JH, Campese VM, et al: Enhanced glucose tolerance in spontaneously hypertensive rats. Pancreatic β-cell hyperfunction with normal insulin sensitivity. Diabetes 41:872-878, 1992

10. Wi JK, Youn JH: Reduced glucose effectiveness is responsible for decreased glucose clearance in STZ diabetic rats. Diabetes 45:250A, 1996 (abstr)

11. Bergman RN, Ider YZ, Bowden CR, et al: Quantitative estimation of insulin sensitivity. Am J Physiol 236:E667-E677, 1979

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

13. Bergman RN, Hope ID, Yang YJ, et al: Assessment of insulin sensitivity in vivo: A critical review. Diabetes Metab Rev 5:411-429, 1989

14. Bergman RN: Toward physiological understanding of glucose tolerance. Minimal-model approach. Diabetes 38:1512-1527, 1989

15. Taniguchi A, Naka Y, Fukushima M, et al: Pathogenic factors responsible for glucose intolerance in patients with NIDDM. Diabetes 41:1540-1546, 1992

16. Martin IK, Weber KM, Boston RC, et al: Effects of epinephrine infusion on determinants of intravenous glucose tolerance in dogs. Am J Physiol 255:E668-E673, 1988

17. Ader M, Pacini G, Yang YJ, et al: Importance of glucose per se to intravenous glucose tolerance. Comparison of the minimal-model prediction with direct measurements. Diabetes 34:1092-1103, 1985

18. Martin IK, Christopher MJ, Alford FP, et al: Distinct but nonadditive effects of epinephrine and cortisol on determinant of glucose tolerance in dogs. Am J Physiol 260:E148-E153, 1991

19. Conway HH, Faas FH, Smith SD, et al: Spontaneous diabetes mellitus in the New Zealand white rabbit: Physiologic characteristics. Metabolism 30:50-56, 1981

20. Kemnitz JH, Rocker EB, Weindruch R, et al: Dietary restriction increases insulin sensitivity and lowers blood glucose in rhesus monkeys. Am J Physiol 266:E540-E547, 1994

21. Clausen JO, Borch-Johnsen K, Ibsen H, et al: Insulin sensitivity index, acute insulin response, and glucose effectiveness in a population-based sample of 380 young healthy caucasians. Analysis of the impact of gender, body fat, physical fitness and life-style factors. J Clin Invest 98:1195-1209, 1996

22. Taniguchi A, Nakai Y, Doi K, et al: Insulin-sensitive and insulin-resistant variants in IGT: A minimal model analysis. Diabetes 43:1211-1217, 1994

23. Buchanan TA, Youn JH, Campese VM, et al: Enhanced glucose tolerance in spontaneously hypertensive rats. Pancreatic β-cell hyperfunction with normal insulin sensitivity. Diabetes 41:872-878, 1992

24. James DE, Burleigh KM, Kraegen EW, et al: Effect of acute exercise and prolonged training on insulin response to intravenous glucose in vivo in rat. J Appl Physiol 55:1660-1664, 1983

25. McBurney MI, Apps KVJ, Finegood DT: Splanchnic infusions of short chain fatty acids do not change insulin sensitivity of pigs. J Nutr 125:2571-2576, 1995

26. Cutfield WS, Bergman RN, Menon RK, et al: The modified minimal model: Application to measurement of insulin sensitivity in children. J Clin Endocrinol Metab 70:1644-1650, 1990

27. Steil GM, Volund A, Kahn SE, et al: Reduced sample number for calculation of insulin sensitivity and glucose effectiveness from the minimal model. Diabetes 42:250-256, 1993

28. Coates PA, Ollerton RL, Luzio SD, et al: Reduced sampling protocols in estimation of insulin sensitivity and glucose effectiveness using the minimal model in NIDDM. Diabetes 42:1635-1641, 1993

29. Yang YJ, Youn JH, Bergman RN: Modified protocols improve insulin sensitivity estimation using the minimal model. Am J Physiol 253:E595-E602, 1987

30. Finegood DT, Hramiak IM, Dupre J: A modified protocol for estimation of insulin sensitivity with the minimal model of glucose kinetics in patients with insulin-dependent diabetes. J Clin Endocrinol Metab 70:1538-1549, 1990

31. Young JC, Garthwaite SM, Bryan JE, et al: Carbohydrate feeding speeds reversal of enhanced glucose uptake in muscle after exercise. Am J Physiol 245:R684-R688, 1983

32. Young DA, Wallberg-Henriksson H, Sleeper MD, et al: Reversal of the exercise-induced increase in muscle permeability to glucose. Am J Physiol 253:E331-E335, 1987

33. Ivy JL, Holloszy JO: Persistent increase in glucose uptake by rat skeletal muscle following exercise. Am J Physiol 241:C200-C203, 1981

34. Richter EA, Garetto LP, Goodman MN, et al: Enhanced muscle glucose metabolism after exercise: Modulation by local factors. Am J Physiol 246:E476-E482, 1984

35. Cartee GD, Young DA, Sleeper MD, et al: Prolonged increase in insulin-stimulated glucose transport in muscle after exercise. Am J Physiol 256:E494-E499, 1989

36. Galante P, Mosthaf L, Kellerer M, et al: Acute hyperglycemia provides an insulin-independent inducer for GLUT4 translocation in C_2C_{12} myotubes and rat skeletal muscle. Diabetes 44:646-651, 1995

37. Printz RL, Magnuson MA, Granner DK: Mammalian glucokinase. Annu Rev Nutr 13:463-496, 1993

38. Meglasson MD, Matschinsky FM: Pancreatic islet glucose metabolism and regulation of insulin secretion. Diabetes Metab Rev 2:163-214, 1986

39. Sweet IR, Peterson L, Kroll K, et al: Effect of glucose on uptake of radiolabeled glucose, 2-DG and 3-*O*-MG by the perfused rat liver. Am J Physiol 271:E384-E396, 1996