Determination and kinetic analysis of non-insulin mediated glucose uptake in Type 1 (insulin-dependent) diabetes mellitus

S. L. Shumak, M. Gulan, B. Zinman and I. S. Gottesman

Department of Medicine, University of Toronto, Toronto, Ontario, Canada

Summary. In man, total glucose uptake is the sum of insulin mediated glucose uptake and non-insulin mediated glucose uptake. The latter pathway has not been examined in Type 1 (insulin-dependent) diabetes mellitus. In order to assess non-insulin mediated glucose uptake in Type 1 diabetes, we measured steady-state rates of glucose uptake during glucose clamps at 5.27, 9.71 and 12.5 mmol/l using low ($0.25 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), intermediate ($0.75 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) and high ($1.50 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) insulin infusion rates in 10 subjects with Type 1 diabetes. For insulin infusion rates of 0.25, 0.75 and $1.50 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ as plasma glucose rose from 5.27 to 9.71 mmol/l, total glucose uptake increased by 35, 43 and 52 percent respectively ($p < 0.05$ for each insulin infusion rate). For all three insulin infusion rates, there was no significant increase in total glucose uptake as plasma glucose increased from 9.71 to 12.5 mmol/l. At each glycaemic level, glucose uptake correlated significantly with plasma free insulin ($r = 0.81$, $p < 0.01$ at 5.71 mmol/l; $r = 0.84$, $p < 0.01$ at 9.71 mmol/l; $r = 0.73$, $p < 0.02$ at 12.5 mmol/l). Linear regression analysis to a point corresponding to plasma free insulin equalling zero, yielded values for non-insulin mediated glucose uptake ($\text{mmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) of 0.11, 0.14, 0.18 at plasma glucose of 5.27, 9.7 and 12.5 mmol/l respectively. Thus, in-

creasing plasma glucose concentrations were associated with increasing rates of non-insulin mediated glucose uptake. For each insulin infusion rate used, the percent of total glucose uptake accounted for by non-insulin mediated glucose uptake remained independent of plasma glucose concentration, but decreased as insulin infusion rate increased. During the insulin infusion at $0.25 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, this percentage ranged from 83.7 to 91.4%. Analysis of glucose uptake data derived for theoretical plasma insulin levels of 0, 40, 80 and $160 \mu\text{U/ml}$ yielded linear Eadie-Hofstee plots ($r = -0.83$ to -0.99), suggesting that insulin increased V_{max} but did not alter K_m . Hence, in these subjects with Type 1 diabetes, glucose uptake, both insulin mediated and non-insulin mediated can be described by Michaelis-Menten kinetics. Comparison of values obtained for V_{max} and K_m in the present studies of Type 1 diabetes with those obtained from non-diabetic subjects indicates that non-insulin dependent glucose uptake in Type 1 diabetes is quantitatively similar to that of non-diabetic subjects.

Key words: Type 1 (insulin-dependent) diabetes, glucose uptake, non-insulin mediated glucose uptake, glucose kinetics.

In man, in vivo glucose uptake is the sum of insulin mediated glucose uptake (IMGU) and non-insulin mediated glucose uptake (NIMGU). IMGU occurs only in insulin sensitive tissues such as muscle and adipose tissue; whereas NIMGU can occur in both insulin sensitive and insulin insensitive tissues (e.g. central nervous system). In the past decade, there have been many investigations of IMGU in man [1]. In contrast, there have been very few investigations of NIMGU de-

spite the fact that non-insulin mediated pathways can account for approximately 70–85% of total glucose uptake in the post-absorptive state. This has been demonstrated in both non-diabetic and Type 2 (non-insulin-dependent) diabetic individuals [2, 3].

The rate of NIMGU maybe a reflection of the activity of the glucose transport system [4]. Defects in this system could contribute to the impairment of insulin action observed in many insulin resistant states including

Table 1. Mean values for plasma glucose and free insulin. G – plasma glucose (mmol/l). FIRI – free plasma immunoreactive insulin ($\mu\text{U}/\text{ml}$)

Insulin infusion rate ($\text{mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)		Target glucose (mmol/l)		
		5.27	9.71	12.5
0.25	G	5.18 ± 0.07	9.83 ± 0.02	12.5 ± 0.07
	FIRI	28.0 ± 8.1	34.2 ± 10.8	39.2 ± 11.7
0.75	G	5.33 ± 0.13	10.16 ± 0.20	12.77 ± 0.17
	FIRI	50.7 ± 6.0	53.5 ± 6.8	54.0 ± 5.5
1.50	G	5.40 ± 0.13	9.60 ± 0.14	12.38 ± 0.07
	FIRI	137 ± 13.7	158 ± 21.0	141 ± 21.0

that reported to be present in Type 1 (insulin-dependent) diabetes mellitus [5, 6]. We therefore sought to study this system in Type 1 diabetes in order to determine whether the apparent abnormality of insulin action present in Type 1 diabetes is secondary to a defect in NIMGU.

Subjects and methods

Subjects

Ten subjects with Type 1 diabetes were studied. Type 1 diabetes diagnosed by the criteria of the National Diabetes Data Group [7] and confirmed by the absence of C-peptide response to an oral meal challenge (Sustacal, Mead-Johnson Co., Belleville, Ontario, Canada) (post-challenge C-peptide $< 0.03 \text{ pmol}/\text{ml}$). Their mean age was 33.7 ± 2.8 years and their duration of diabetes was 14.8 ± 2.4 years. Subjects averaged $103 \pm 2\%$ of their predicted ideal body weight according to estimates obtained using the Metropolitan Life tables of 1959. Subjects used 42.8 ± 5.6 units of insulin per day ($0.63 \pm 0.08 \text{ units} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$). Seven subjects took twice daily insulin injections, 1 thrice daily and 2 subjects took one injection daily. No subject had clinically significant diabetic complications. The mean haemoglobin A1c was $7.2 \pm 0.5\%$ (normal range 3–6%). No attempt was made to alter the glycaemic control in our subjects prior to acceptance into this study.

Procedures

Upon entry into the study, each subject was randomised to undergo three glucose clamps at one of three insulin infusion rates (0.25, 0.75 or $1.50 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). In the text, these rates are designated as low, intermediate and high respectively. Two subjects volunteered for further clamps and were assigned randomly to one of the remaining insulin infusion rates for a series of three further clamp studies.

Intermediate acting insulin was discontinued 72 h before each clamp study, and replaced by thrice daily injections of regular insulin until 12 h prior to each clamp. At that time, a continuous intravenous infusion of regular insulin (Iletin II, Eli Lilly Co., Indianapolis, Ind., USA) was given to achieve euglycaemia overnight (plasma glucose 5–6 mmol/l) according to a modification of a previously published protocol [8]. This infusion was continued until the start of the clamp period the next morning and then discontinued. All studies began at 08.00 hours with the subject in the post-absorptive state. Female subjects underwent each study in the follicular phase of their menstrual cycle. The purpose, nature and risks of the investigation were fully explained to the participants and written informed consent was obtained. The protocol was approved by the Human Studies Committee of the University of Toronto.

Glucose clamps

Volunteers underwent three glucose clamps, each of 4 h duration, with target glycaemic levels of 5.27, 9.71 and 12.5 mmol/l. For a given volunteer, the same insulin infusion rate was used in all three clamps. For all subjects the order of target glucose levels was selected randomly, and the interval between clamp studies ranged from two to four weeks.

Glucose clamping was performed as previously described [9]. At the start of the clamp, a primed ($22 \mu\text{Ci}$) continuous ($0.22 \mu\text{Ci}/\text{min}$) infusion of 3-[3H] glucose (New England Nuclear, Boston, Mass., USA) was begun. Concurrently, infusions of Somatostatin (Serono, Randolph, Mass., USA) at $250 \mu\text{g}/\text{h}$ and regular pork insulin (Iletin II, Eli Lilly) at one of 0.25, 0.75 or $1.50 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ were begun. A priming dose of insulin was given intravenously over 10 s (and calculated according to the formula: insulin prime (mU) = distribution volume ($100 \text{ ml}/\text{kg} \times \text{body weight in kg}$) $\times$ desired increment in plasma insulin ($\mu\text{U}/\text{ml}$) $\times 0.001$). A basal plasma insulin concentration of $10 \mu\text{U}/\text{ml}$ and an insulin distribution volume of 10% body weight were assumed [10]. Simultaneous with these infusions, a fifty percent solution of glucose was infused at a variable rate in order to maintain the plasma glucose at a constant level (5.27, 9.71 or 12.5 mmol/l). In order to prevent hypokalaemia and hypophosphataemia, a carrier solution of 0.9 percent NaCl containing 0.13 meq/ml potassium and 0.09 mmol/l phosphate was administered intravenously at 30 ml/h.

Analytical methods

All blood samples were drawn from an arterialed (70°C) hand vein [11]. Plasma glucose and urine glucose were measured using the glucose oxidase method (Glucose Analyser II, Beckman Instruments, Fullerton, Calif, USA). Blood for determination of free insulin was drawn into tubes containing 1000 U/ml aprotinin (Trasylol, Miles Laboratories, Rexdale, Ont., Canada) and placed on ice. Samples were centrifuged with minimal delay and the supernatants frozen at -20°C until assayed. Following polyethylene glycol extraction of the serum [12], free insulin was determined by radioimmunoassay using a single antibody charcoal separation method [13]. Blood for glucose specific activity was drawn into tubes containing NaF-oxalate and placed on ice. Following centrifugation, glucose specific activity in the supernatant was determined as previously described [14].

Glucose uptake rates

Glucose specific activity was determined on timed samples taken during the last 30 min of each glucose clamp. Glucose uptake was then determined isotopically using the equations of DeBodo et al. [15]. These have been shown to accurately reflect glucose kinetics over a wide range of non-steady-state conditions [16, 17]. A pool fraction of 0.5 was assumed. Calculated glucose uptake rates were corrected for urine glucose losses.

Table 2. Rates of total glucose uptake (Rd) and percent of Rd accounted for by non-insulin mediated glucose uptake (%NIMGU) at the target glucose levels used in this study

Insulin infusion rate (mU·kg ⁻¹ ·m ⁻¹)	Rd (mmol·kg ⁻¹ ·m ⁻¹)			%NIMGU		
	5.27	9.71	12.5	5.27	9.71	12.5
0	0.11	0.14	0.18	-	-	-
0.25	0.12 ± 0.01	0.16 ± 0.02	0.22 ± 0.04	91.7	87.1	83.4
0.75	0.24 ± 0.03	0.34 ± 0.07	0.37 ± 0.08	46.6	41.6	48.8
1.50	0.43 ± 0.04	0.66 ± 0.05	0.69 ± 0.06	25.8	21.8	26.2

Statistical analysis

All calculations were made on mean steady-state data collected over the last 30 min of each clamp. Least squares linear regression analysis was performed on the data obtained during the 5.27, 9.71, 12.5 mmol/l clamps, respectively. Glucose uptake was considered as the dependent variable and plasma free insulin level as the independent variable. For each glycaemic level, the 'y' intercept (i.e. free insulin = 0) of the regression line was taken to represent NIMGU at that level [20]. Using these regression equations, values of glucose uptake were derived for theoretical plasma free insulin levels of 0, 40, 80 and 160 µU/ml. Eadie-Hofstee analysis of the glucose uptake data at these insulin levels was then performed. The percent of total glucose uptake attributable to NIMGU (%NIMGU) was defined as $100 \times \text{NIMGU} / \text{total glucose uptake}$. Statistical comparison of rates of glucose uptake at various plasma glucose and plasma free insulin concentration were made using paired and unpaired Student's t-tests. Comparisons of multiple means was made using one way analysis of variance (F test). All data in the text, tables and figures is given as mean ± SEM.

Results

Target glycaemic level was reached after 24.6 ± 4.9 , 21.1 ± 3.1 and 24.0 ± 3.2 min for clamps with glucose goals of 5.27, 9.71, and 12.5 mmol/l respectively. Pre-clamp glucose was 5.71 ± 0.38 , 5.50 ± 0.27 and 7.14 ± 0.66 for clamps with glucose goals of 5.27, 9.71 and 12.5 mmol/l respectively. The mean values for plasma glucose and free insulin during the study are listed in Table 1. For each insulin infusion rate, the free plasma insulin levels attained were similar for clamps with target glucose of 5.27, 9.71 and 12.5 mmol/l (pNS by F test). As well for each target glycaemic level, the glucose concentrations achieved during the clamps at the three insulin infusion rates were not statistically different (pNS by F test).

At plasma glucose of 5.27, 9.71 and 12.5 mmol/l respectively, glucose uptake (mmol·kg⁻¹·min⁻¹) was 0.12 ± 0.01 , 0.16 ± 0.02 and 0.22 ± 0.04 at the low insulin infusion rate, 0.24 ± 0.03 , 0.34 ± 0.07 and 0.37 ± 0.08 at the intermediate insulin infusion rate, and 0.43 ± 0.04 , 0.66 ± 0.05 and 0.69 ± 0.06 at the high insulin infusion rate (Table 2). Thus, increasing plasma glucose concentration from 5.27 to 9.71 at the low, intermediate and high insulin rates was associated with increases in glucose uptake of 35, 43 and 52%, respectively ($p < 0.05$).

Fig. 1. The relationship between glucose uptake and plasma glucose during insulin infusion rates of 1.50 (Δ), 0.75 (□) and 0.25 (○) mU·kg⁻¹·min⁻¹. * $p < 0.05$ vs. glucose uptake at 5.27 mmol/l for same insulin infusion rate

For all insulin infusion rates used, the change in glucose uptake associated with increasing plasma glucose from 9.71 to 12.5 mmol/l was not significant (Fig. 1).

Figures 2a, b and c depict the relationship between glucose uptake and plasma free insulin levels during clamps with target glycaemic levels of 5.27, 9.71 and 12.5 mmol/l, respectively. In each case, a significant correlation was observed ($r = 0.81$, $p < 0.01$; $r = 0.84$, $p < 0.01$; $r = 0.73$, $p < 0.02$ for plasma glucose 5.27, 9.71 and 12.5 mmol/l, respectively). Linear regression analysis to free plasma insulin concentration equalling zero yielded values for NIMGU of 0.11, 0.14 and 0.18 mmol·kg⁻¹·min⁻¹ at plasma glucose equalling 5.27, 9.71 and 12.5 mmol/l respectively. Table 2 lists values for total glucose uptake and %NIMGU for the plasma free insulin and glucose levels achieved in this study. For a given insulin infusion rate, the %NIMGU was independent of plasma glucose concentration. However, the %NIMBU decreased as insulin infusion rate increased.

Results of Eadie-Hofstee analysis of the glucose uptake data are illustrated in Figure 3. For each insulin level analysed, a strong correlation was obtained ($r = -0.83$ to -0.99). In this analysis, the 'y' intercept is

Fig. 2. **a** The relationship between glucose uptake and plasma free immunoreactive insulin at a glucose concentration of 5.27 mmol/l. The least mean squares linear regression line is shown ($y = 0.111 + 0.00219x$, $r = 0.81$, $p < 0.01$). **b** The relationship between glucose uptake and plasma free immunoreactive insulin at a glucose concentration of 9.71 mmol/l. The least mean squares linear regression line is shown ($y = 0.142 + 0.00306x$, $r = 0.84$, $p < 0.01$). **c** The relationship between glucose uptake and plasma free immunoreactive insulin at a glucose concentration of 12.5 mmol/l. The least mean squares linear regression line is shown ($y = 0.181 + 0.00324x$, $r = 0.73$, $p < 0.02$)

Fig. 3. Eadie-Hofstee analysis of glucose uptake at free insulin of 0 (●—●), 40 (○—○), 80 (■—■) and 160 (△—△) μU/ml

the V_{max} and the negative slope is K_m . Hence, it can be seen that with the increasing insulin concentration the V_{max} progressively increased. K_m was unchanged (7.8, 8.2, 8.1, 7.9 mmol/l) at insulin concentration of 0, 40, 80, and 160 μU/ml.

Discussion

Glucose uptake by mammalian cells is thought to occur via glucose transporters located in the cell membrane [18, 19]. In vitro studies have shown that insulin causes translocation of these glucose transporters from a cytoplasmic pool to the cell membrane [18]. Thus, in insulin sensitive tissues, insulin could augment glucose uptake by providing additional glucose transporters. In vivo data obtained from non-diabetic subjects has demonstrated that glucose uptake follows Michaelis-Menten kinetics, with insulin increasing the V_{max} but not changing the K_m [20]. This finding has been interpreted as indicating that insulin increases the number of effective glucose transporters without changing their affinity for glucose [4, 20].

Glucose transport occurring independently of insulin action may reflect the intrinsic activity of the glucose transporter system [4]. A defect in this intrinsic activity could impair glucose uptake even after insulin has induced translocation of cytoplasmic transporters to the cell membrane. With many currently employed techniques for assessing insulin resistance, such an impairment might be taken, incorrectly, to indicate a primary defect in insulin action [1, 4]. In this investigation, we studied NIMGU in subjects with Type 1 diabetes in order to determine if the insulin resistance of Type 1

diabetes could be explained by abnormalities in NIMGU.

In this study, our measurement of NIMGU was made indirectly by extrapolation of measured values for glucose uptake at non-zero insulin levels. This derivation depends on the assumption that glucose uptake continues to vary linearly with the plasma insulin level at low plasma insulin concentration. Two lines of evidence support this assumption. In vivo, glucose uptake varies linearly with plasma insulin between insulin concentrations of 1 to 46 $\mu\text{U/ml}$ [2, 21]. In vitro, glucose uptake has also been shown to be linear over insulin concentrations ranging from 0 to 25 $\mu\text{U/ml}$ [22–24]. As has been demonstrated previously [2], it thus seems reasonable to assume that glucose uptake in vivo varies linearly with plasma insulin even at low plasma insulin concentrations.

We have demonstrated that in Type 1 diabetes glucose uptake, both insulin mediated and non-insulin mediated can be described by Michaelis-Menten kinetics. Specifically, we observed a linear relationship between glucose uptake and glucose uptake/plasma glucose (Eadie Hofstee analysis) for plasma insulin concentrations of 0, 40, 80 and 160 $\mu\text{U/ml}$. Insulin increased the V_{max} for glucose uptake without changing the K_{m} . This characteristic of glucose uptake has been described previously in non-diabetic subjects [20], where it was interpreted to mean that insulin augments glucose transport solely by increasing the number of effective glucose transporters.

Table 3 summarises values for V_{max} and K_{m} for glucose uptake in the present study and values reported for non-diabetic subjects [2]. Values for V_{max} are somewhat lower for the Type 1 diabetic subjects, especially at the higher insulin levels. This may be explained by the fact that at the higher insulin concentrations the %NIMGU is less (Table 2), and the majority of glucose uptake occurs through insulin mediated mechanisms. The values for K_{m} for glucose uptake for both Type 1 diabetes and non-insulin subjects are similar, suggesting that the glucose transport system is intact in these subjects with Type 1 diabetes. Of note is the fact that our research subjects had an average haemoglobin A1c of 7.2% (normal less than 6%). Thus they were in relatively good glycaemic control. It is, therefore, possible that the glucose transport system is still normally operative in well controlled Type 1 diabetic subjects whereas this may not be demonstrated in those subjects in poorer glycaemic control. In addition, it must be remembered that those subjects with a haemoglobin A1c that is exceedingly elevated may have other abnormalities of intermediary metabolism that would interfere with glucose metabolism and hence the results and interpretations.

In the post-absorptive state in non-diabetic subjects, NIMGU accounts for approximately 75 to 85% of total glucose uptake [2]. In Type 1 diabetes, the corresponding figure has been reported to be 71% [3].

Table 3. Values for K_{m} and V_{max} obtained in non-diabetic subjects (2) and the present study. FIRI – free immunoreactive insulin ($\mu\text{U/ml}$)

FIRI	K_{m} (mmol/l)		V_{max} (mmol $\cdot$ kg $^{-1}$ $\cdot$ m $^{-1}$)	
	Non-diabetic subjects	Type 1 diabetic patients	Non-diabetic subjects	Type 1 diabetic patients
0	9.9	7.8	0.29	0.27
20	9.3	8.2	0.36	0.39
80	11.9	8.1	1.03	0.71
160	10.3	7.9	1.46	1.13

In our subjects with Type 1 diabetes, we have shown that approximately 85 to 90% of glucose uptake is non-insulin-mediated during the low insulin infusion rate (plasma insulin concentrations 28–39 $\mu\text{U/ml}$). These plasma insulin levels were higher than those present in the basal state (after overnight euglycaemia, pre-clamp). Thus, the %NIMGU in our subjects in the post-absorptive state may have been slightly greater than 85–90%. However, the fact that NIMGU in Type 1 diabetes is quantitatively similar to that in non-diabetic subjects reported in the literature to suggests that abnormalities of insulin sensitivity in Type 1 diabetes may be secondary to defective insulin action and are not due to an irreversible defect in the glucose transport system.

Acknowledgements. These studies were supported by the Canadian Diabetes Association and Mount Sinai Hospital Research Fund (ISG). In addition, S. L. S. was the Connaught Novo Research Fellow of the Canadian Diabetes Association. We thank B. E. Noble, RN whose dedication made these studies possible and the technical staff of the Banting and Best Diabetes Core Laboratory. We thank Ms. M. Marczak and Ms. D. Orlans for secretarial assistance.

References

1. Bergman RN, Finegood DT, Ader M (1985) Assessment of insulin sensitivity in vivo. *Endocr Rev* 6: 45–86
2. Gottesman I, Mandarino L, Gerich J (1983) Estimation and kinetic analysis of insulin-independent glucose uptake in human subjects. *Am J Physiol* 244: E632–T635
3. Baron AD, Kolterman OG, Bell J, Mandarino LJ, Olefsky JM (1985) Rates of noninsulin-mediated glucose uptake are elevated in type II diabetic subjects. *J Clin Invest* 76: 1782–1788
4. Gottesman I, Mandarino L, Gerich J (1984) Use of glucose uptake and glucose clearance for the evaluation of insulin action in vivo. *Diabetes* 33: 184–191
5. DeFronzo RA, Hendler R, Simonson D (1982) Insulin resistance is a prominent feature of insulin-dependent diabetes. *Diabetes* 31: 795–801
6. Yki-Jarvinen H, Koivisto VA (1986) Natural course of insulin resistance in type I diabetes. *N Engl J Med* 315: 224–230
7. National Diabetes Data Group (1979) Classification and diagnosis of diabetes mellitus and other categories of glucose tolerance. *Diabetes* 28: 1039–1057
8. White NH, Skor D, Santiago JV (1982) Practical closed-loop insulin delivery. *Ann Int Med* 97: 210–213
9. Rizza R, Mandarino L, Gerich J (1981) Dose-response character-

- istics for the effect of insulin on production and utilization of glucose in man. *Am J Physiol* 240: E630-E639
10. Sherwin RS, Kramer KJ, Tobin JD, Insel PA, Liljen Quist JE, Berman M, Andres R (1974) A model of the kinetics of insulin in man. *J Clin Invest* 53: 1481-1492
11. McGuire E, Helderman J, Tobin J, Andres R, Berman M (1976) Effects of arterial versus venous sampling on analysis of glucose kinetics in man. *J Appl Physiol* 41: 565-573
12. Desbuquois B, Auerbach GD (1971) Use of polyethylene glycol to separate free and antibody-bound peptide hormones in radioimmunoassays. *J Clin Endocrinol Metab* 33: 732-738
13. Hanna AK, Zinman B, Nakhooda AF, Minuk HL, Stokes EF, Albisser AM, Leibel BS, Marliss EB (1980) Insulin, glucagon and aminoacids during glycemic control by the artificial pancreas in diabetic man. *Metabolism* 29: 321-332
14. Rizza R, Mandarino L, Gerich J (1981) Mechanism and significance of insulin resistance in noninsulin-dependent diabetes mellitus. *Diabetes* 30: 990-995
15. DeBodo R, Steele R, Alstszuler N, Dunn A, Bishop J (1963) On the hormonal regulation of carbohydrate metabolism: studies with C14-glucose. *Recent Prog Res* 19: 445-488
16. Dunn A, Katz J, Golden S, Chenoweth M (1976) Estimation of glucose turnover and recycling in rabbits using various [3H14C] glucose labels. *Am J Physiol* 230: 1159-1162
17. Radziuk J, Norwich KH, Vranic M (1978) Experimental validation of measurements of glucose turnover in nonsteady state. *Am J Physiol* 234: E84-E93
18. Cushman S, Wardzala L (1980) Potential mechanism of insulin action on glucose transport in the isolated rat adipose cell. Apparent translocation of intracellular transport systems to the plasma membrane. *J Biol Chem* 255: 4758-4762
19. Karnielli E, Hissin P, Simpson I, Salans L, Cushman S (1981) A possible mechanism of insulin resistance in the rat adipose cell in streptozotocin-induced diabetes mellitus. *J Clin Invest* 68: 811-814
20. Gottesman I, Mandarino L, Verdonk C, Rizza R, Gerich J (1982) Insulin increases the maximum velocity for glucose uptake without altering the Michaelis constant in man. *J Clin Invest* 70: 1310-1314
21. Best JD, Taborsky GJ Jr, Halter JB, Porte D Jr (1981) Glucose disposal is not proportional to plasma glucose level in man. *Diabetes* 30: 847-850
22. Ciaraldi T, Kolterman O, Siegal J, Olefsky J (1979) Insulin-stimulated glucose transport in human adipocytes. *Am J Physiol* 236: E621-E625
23. LeMarchand-Brustel Y, Freychet P (1979) Effect of fasting and streptozotocin diabetes on insulin binding and action in the isolated mouse soleus muscle. *J Clin Invest* 64: 1505-1515
24. Berhanu P, Olefsky J (1981) Effects of insulin and insulin-like agents on the glucose transport system of cultured human fibroblasts. *Diabetes* 30: 523-529

Received: 9 May 1988
and in revised form: 23 November 1988

Dr. I. Gottesman
2300 Eglinton Avenue West
Suite 506
Mississauga, Ontario
L5M 2V8
Canada