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Research Article

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Rates of Noninsulin-mediated Glucose Uptake Are Elevated in Type II Diabetic Subjects

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Abstract

Although insulin is extremely potent in regulating glucose transport in insulin-sensitive tissues, all tissues are capable of taking up glucose by facilitated diffusion by means of a noninsulin-mediated glucose uptake (NIMGU) system. Several reports have estimated that in the postabsorptive state the majority of glucose disposal occurs via a NIMGU mechanism. However, these estimates have been either derived or extrapolated in normal humans. In the present study we have directly measured NIMGU rates in 11 normal (C) and 7 Type II noninsulin-dependent diabetic subjects (NIDDM; mean±SE fasting serum glucose, 249±24 mg/dl). To accomplish this, the serum glucose was clamped at a desired level during a period of insulin deficiency induced by a somatostatin infusion (SRIF, 550 μg/h). With a concomitant [3-^{3}H]glucose infusion, we could isotopically quantitate glucose disposal rates (R_d) during basal (basal insulin present) and insulin-deficient (SRIF) conditions. With this approach we found that (a) basal R_d was greater in NIDDM than in C, 274±31 vs. 150±7 mg/min, due to elevated hepatic glucose output, (b) NIMGU composes 75±5% of basal R_d in C and 71±4% in NIDDM, (c) NIDDMS have absolute basal NIMGU rates that are twice that of C (195±23 vs. 113±8 mg/min, $P < 0.05$), (d) when C were studied under conditions of insulin deficiency (SRIF infusion) and at a serum glucose level comparable to that of the NIDDM group (250 mg/dl), their rates of NIMGU were the same as that of the NIDDM group (186±19 vs. 195±23 mg/min; NS). We conclude that (a) in the postabsorptive state, NIMGU is the major pathway for glucose disposal for both C and NIDDM; (b) for a given glucose level the efficiency of NIMGU (NIMGU ÷ serum glucose level) is equal in C and NIDDM, but since basal R_d is elevated in NIDDMs their absolute basal rates of NIMGU are higher; and (c) elevated basal rates of NIMGU in NIDDM may play a role in the pathogenesis of the late complications of diabetes.

Introduction

In vivo glucose uptake can occur via two mechanisms, namely, insulin-mediated glucose uptake (IMGU)[1] and noninsulin-me-

1. *Abbreviations used in this paper:* CNS, central nervous system; FFA, free fatty acid; IMGU, insulin-mediated glucose uptake; NIDDM, noninsulin-dependent diabetic subjects; NIMGU, noninsulin-mediated glucose uptake; R_a, rate of glucose appearance; R_d, rate of glucose disappearance; SRIF, somatostatin.

diated glucose uptake (NIMGU). By definition, IMGU can occur only in insulin-sensitive tissues. On the other hand, NIMGU occurs in both insulin-sensitive and noninsulin-sensitive tissues. Although the literature abounds with reports of insulin-stimulated glucose disposal rates (IMGU) measured under different conditions and in different experimental groups, there is a paucity of literature relating to noninsulin-mediated glucose uptake in vivo (1, 2). This is surprising, since it has been estimated that the majority of glucose disposed of in the basal state is through NIMGU mechanisms (3, 4). Gottesman et al. (2) have estimated that in normal human subjects 75–85% of postabsorptive glucose uptake is noninsulin-mediated. This estimate was obtained by measuring glucose uptake isotopically while maintaining the serum glucose constant over a range of insulin levels. Glucose uptake at "0" insulin was estimated by plotting glucose disposal rates vs. plasma insulin concentrations and extrapolating to the intercept for glucose disposal at "0" insulin. This indirect method assumes linearity of glucose uptake, over insulin concentrations lower than those actually employed in the study, and therefore may not accurately reflect true rates of NIMGU.

Elucidation of the NIMGU system is important, as it is likely to be the major pathway of glucose uptake in the basal state. Furthermore, knowledge of NIMGU rates would allow for more precise estimates of strictly insulin-stimulated glucose uptake rates. Since overall insulin-stimulated glucose disposal, in fact, represents the sum of NIMGU and IMGU, if one had knowledge of that portion of overall insulin-stimulated glucose disposal which was NIMGU, then the difference would represent true insulin-stimulated glucose disposal. In addition, though it is well recognized that Type II diabetes is characterized by a marked impairment in insulin-stimulated glucose uptake, the status of NIMGU mechanisms in Type II diabetics is unknown.

Therefore, this study was undertaken to directly measure NIMGU in normal and Type II diabetic subjects over a large range of glucose concentrations. To accomplish this, glucose clamp studies were performed under conditions of insulin deficiency induced by a somatostatin infusion and glucose uptake rates determined isotopically. Each study was performed at each subject's respective fasting serum glucose level; control subjects were also studied under hyperglycemic conditions comparable to the diabetic group.

Methods

Materials. Porcine monocomponent insulin was generously supplied by Dr. Ronald Chance of Eli Lilly and Co. (Indianapolis, IN); ^{125}I-Insulin and [3-^{3}H]glucose were purchased from New England Nuclear (Boston, MA); bovine serum albumin (fraction V) was obtained from Armour Pharmaceutical Company (Tarrytown, NY); guinea pig anti-insulin antibody was kindly supplied by Dr. Edward Arquilla (Irvine, CA); somatostatin (cyclic form) was purchased from Bachem Incorporated (Torrance, CA).

Subjects. The study group consisted of 11 control subjects (normal OGTT) and 7 subjects with Type II diabetes mellitus as defined by the criteria of the National Diabetes Data Group (5). The clinical and met-

abolic characteristics of the subjects are summarized in Table I. The fasting serum glucose and insulin levels represent the mean of five determinations done on consecutive days. The mean age±SEM of the diabetic subjects was 38±4 yr compared with 34±2 yr for the normal subjects, P = NS. The relative weights of the Type II diabetics ranged from 0.92 to 1.23 and from 0.77 to 1.16 in the control group; with a mean±SEM value of 1.07±0.14 and 0.99±0.33 for the diabetic and control subjects, respectively; P = NS. The mean weight±SEM was 77±5 and 63±3 kg in the diabetic group and control group, respectively; P < 0.05. After informed consent was obtained, all subjects were admitted to the University of California, San Diego, General Clinical Research Center, where they remained active to approximate their prehospital exercise level. Diabetic subjects were withdrawn from insulin for at least 10 d and from sulfonylureas for 3 wk before study. All subjects were chemically euthyroid and no subject had a concurrent disease or was ingesting pharmacological agents known to affect carbohydrate or insulin metabolism.

Diet. All subjects were placed on a weight-maintenance (32 Kcal/kg per d) liquid formula diet, with three divided feedings containing 1/5, 2/5, and 2/5 of the total daily calories given at 8 a.m., 12 a.m., and 5 p.m., respectively. The diet contained 45% carbohydrate, 40% fat, and 15% protein. All subjects equilibrated on this diet for at least 48 h before any studies were performed. Each study was performed on separate but consecutive days.

Oral glucose tolerance test. All control subjects underwent a 75-g oral glucose tolerance test after an overnight fast. Serum was obtained at 0, 30, 60, 120, and 180 min for measurement of glucose and insulin levels.

Somatostatin (SRIF) and glucose clamp studies. During each study, SRIF dissolved in 0.9% normal saline to which 2 ml of patient's own serum was added and was infused at a rate of 550 μg/h starting at time 0 in both control and Type II diabetic (NIDDM) groups with no insulin replacement. In the control group, each subject underwent a 5-h glucose clamp study with the serum glucose held at or near the fasting level, by a variable rate exogenous glucose infusion. In addition, seven control subjects underwent a 4-h hyperglycemic clamp study with the serum glucose acutely raised and held at a mean level of 248±2 mg/dl (the mean fasting serum glucose for the NIDDM group). In the NIDDM group, each subject underwent a 5-h clamp study with the glucose level held at their respective fasting level (mean±SE, 253±24 mg/dl; range, 163–348 mg/dl).

Glucose disposal rates. R_d, the rate of overall glucose disappearance, and R_a, the rate of glucose appearance, were quantitated in the basal state and during each of the glucose clamp studies (6) by infusing [3-^{3}H]glucose in a primed, continuous manner. With this technique, 50 μCi of the tracer is injected as a bolus, followed by a continuous infusion at the rate of 0.50 μCi/min. After a 60-min equilibration period, blood samples were obtained at 20-min intervals, to determine both the serum glucose concentration and plasma specific activity. R_a and R_d were calculated with the Steele equations in their modified derivative form, since the tracer exhibits nonsteady-state kinetics under these conditions (7). The values for R_d in the basal state and during the hyperglycemic studies were corrected for urinary glucose loss, to reflect the actual rate of endogenous glucose disposal.

Table I. Patient Characteristics

	Age	Relative body weight	Weight	Fasting serum glucose	Fasting serum insulin	Fasting serum C-peptide
	yr		*kg*	*mg/dl*	*μu/ml*	*pmol/ml*
Control						
n = 11	34±2	0.99	63±3	90±1.7	9.0±1.1	0.55±0.06
Diabetic						
n = 7	38±4	1.07	77±5*	249±24‡	17±3§	1.41±0.22§

Data are represented as mean±SEM.
* P < 0.05; ‡ P < 0.001; § P < 0.01.

Rates of NIMGU were measured during the period of SRIF-induced insulin deficiency, while the serum glucose was maintained at basal levels by a variable exogenous glucose infusion. Under these conditions, the maintenance of a steady-state serum glucose level is a result of several events (8), which should be noted. Thus, within 20 min of the onset of the SRIF infusion, R_a falls due to glucagon deficiency (data not shown); at the same time, the SRIF-induced hypoinsulinemia causes a decline in R_d. However, due to relatively slow decay in insulin action, R_d declines more slowly than R_a, and this imbalance results in a fall in the serum glucose level. To prevent this fall and maintain the serum glucose at the basal level, it is necessary to infuse glucose exogenously. At approximately 180 min, R_a progressively recovers (data not shown) and eventually approaches or exceeds R_d. Despite the discontinuation of the exogenous glucose infusion, this can lead to an overshoot in the serum glucose level above base line, and this phenomenon is most evident at 220 min in the control group (Fig. 1, top left panel). It was therefore not possible in all cases to "clamp" the serum glucose continuously at the basal value in the latter part of some of the control studies. In the NIDDM group, R_a and R_d fell during the initial part of the study, but recovery of R_a was not as brisk and we were able to better compensate by decreasing the exogenous glucose infusion and avoid a rise in the serum glucose level above basal (Fig. 1, top right panel). Thus, to calculate steady-state rates of NIMGU, we used the data obtained during time intervals at which the serum glucose level (101 mg/dl) was within 12% of the basal value in the control group (between 120 and 200 min) and within 5% (260 mg/dl) of the basal value in the NIDDM group (between 120 and 200 min). During these time intervals, steady-state conditions are exhibited by the stable serum glucose level and stable glucose disposal rates R_d (Fig. 1, bottom). As previously stated, NIMGU can occur in insulin-sensitive and nonsensitive tissues. However, it has been estimated that the great majority of NIMGU occurs in the central nervous system (CNS) (3–4), whereas muscle accounts for the preponderance of IMGU (9). Since the CNS accounts for most of NIMGU, it follows that NIMGU will not increase in proportion to overall body mass. Thus, it would seem incorrect to normalize rates of NIMGU to some aspect of body size or mass. On the other hand, since IMGU occurs predominantly in the muscle, it seems reasonable to normalize this measurement to some aspect of body mass. Based on this, we chose to express rates of NIMGU in mg/min, and IMGU as mg/kg per min.

Analytical methods. Blood for serum glucose determinations was drawn, placed in untreated polypropylene tubes, and spun using a microfuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, CA). The glucose concentration of the supernatant was then measured by the glucose oxidase method using a glucose analyzer (Yellow Springs Instrument Co., Yellow Springs, OH). Blood for determination of serum insulin levels and plasma glucose specific activity was collected in treated and untreated tubes, respectively, and allowed to clot. The specimens were spun and the serum was removed and stored at $-20°$C. Serum-free insulin levels and total insulin levels were measured by double antibody radioimmunoassay (10). NIDDM subjects with a history of previous insulin injections and in whom insulin antibodies were detected, had serum-free insulin levels determined. Blood for determination of serum C-peptide levels was collected in tubes containing EDTA and Trasylol (500 U/ml) and placed on ice; the serum was separated and frozen. The C-peptide radioimmunoassay was kindly performed in Dr. Arthur Rubenstein's laboratory (Chicago, IL).

Data analysis. All calculations and analysis were performed using the CLINFO system of the University of California, San Diego General Clinical Research Center. Data presented represent the mean (±SEM) unless otherwise stated. Statistical analysis was done using the two-tailed Student's t test for paired data and unpaired data and the Wilcoxon signed rank test for nonparametric data as indicated.

Results

Serum glucose and hormone levels. The mean fasting serum glucose and insulin levels were 90±2 mg/dl and 9±2 μU/ml in

controls, and 249±24 mg/dl and 17±3 μU/ml in the NIDDM subjects, respectively (Table I). Basal C-peptide levels were 0.55±0.06 in the control and 1.41±0.22 pmol/ml in the NIDDM group, $P < 0.01$ (Table I). During the SRIF infusion the serum insulin level dropped rapidly from basal in both groups and within the first hour, steady-state insulin levels were below the detectable limits of the assay (<4 μU/ml). Steady-state C-peptide measurements revealed a >92% reduction from basal levels during the SRIF infusion, decreasing to levels of 0.025±0.002 pmol/ml in controls and 0.10±0.01 pmol/ml in NIDDM. The value in the NIDDM group was still significantly greater than control, $P < 0.001$. Thus, SRIF induced a profound and sustained state of severe insulin deficiency in the peripheral circulation. Since the biologic effect of insulin to stimulate glucose disposal decays with an apparent half-time of ~40 min (11), it follows that after 120 min of absolute insulin deficiency, isotopically determined glucose disposal rate (R_d) represents NIMGU.

Basal glucose disposal rates (basal insulin present). In the basal state, the serum glucose level is quite stable; therefore, the rate of overall glucose appearance R_a (hepatic glucose production) must equal the rate of glucose utilization (R_d). Because of prevailing hyperglycemia, some NIDDM subjects will exhibit glucosuria and R_d must be corrected for urinary losses in order to reflect only tissue glucose disposal. Despite this correction, the basal rate of overall glucose disposal (NIMGU + IMGU) was greatly increased in NIDDM compared with control subjects, 274±31 vs. 150±7 mg/min, respectively ($P < 0.01$, Fig. 1, bottom).

As can be seen in Fig. 2 *A*, the mean rate of NIMGU was 113±8 mg/min in controls, when measured at euglycemia, compared with 195±23 mg/min in NIDDMs when measured at about the mean fasting serum glucose of 249±24 mg/dl. Since these rates were measured at basal glucose levels for both groups, they represent the amount of basal R_d accounted for by NIMGU. Therefore, the NIDDM subjects have basal rates of NIMGU that are ~2 times that of control subjects.

Since basal R_d (basal insulin present) was measured in both groups (Fig. 2 *B*), we were also able to calculate the proportion of basal R_d that occurs via noninsulin-mediated mechanisms; ~75±5% of the basal R_d is NIMGU in control and 71±4% in

NIDDM subjects (Fig. 2 *C*). Thus, even though absolute rates of NIMGU are markedly increased in NIDDMs, NIMGU composes approximately the same proportion of basal R_d in both groups.

If overall basal R_d (NIMGU + IMGU) and basal NIMGU are known, then IMGU can be calculated (IMGU = R_d − NIMGU). Absolute rates of basal IMGU were higher in NIDDM than in control (1.0±0.24 vs. 0.70±0.3 mg/kg per min); however, these differences did not reach statistical significance ($P = 0.21$; Fig. 2 *D*).

To directly compare NIMGU in control and NIDDM at a similar serum glucose level, we studied seven control subjects under hyperglycemic conditions. This was done by acutely raising the serum glucose to a mean level of 248±2 mg/dl (comparable to the mean fasting serum glucose level found in the NIDDM group) via a variable exogenous glucose infusion (Fig. 3). Again, SRIF was infused to suppress endogenous insulin release and NIMGU was measured isotopically. At all times the serum insulin levels remained suppressed to a similar degree as in the euglycemic studies, and exhibited no breakthrough; steady-state C-peptide levels were reduced by 85% of the basal value, to a mean steady-state level of 0.09±0.02 pmol/ml. To acutely raise the serum glucose level, large amounts of glucose were infused over the first 60 min, and as the serum glucose approached the desired level the glucose infusion was decreased to match the rate of glucose disposal and achieve new steady-state conditions. By 120 min the serum glucose was stable and R_d approximated R_a; therefore, the data between 120–200 min were used to calculate NIMGU. Under these conditions (serum glucose level = 250 mg/dl), the rate of NIMGU in the control group was 186±19 mg/min.

Since NIMGU was estimated under similar conditions and at a comparable mean glucose level (~250 mg/dl) in control and NIDDM subjects, we can directly compare NIMGU rates in both groups. As can be seen in Fig. 4, at a serum glucose of ~250 mg/dl, NIMGU was quite similar for both NIDDM and control groups, 197±14 mg/min and 186±19 mg/min, respectively, P = NS. This indicates that at a serum glucose level of 250 mg/dl, NIMGU functions at normal efficiency in NIDDM.

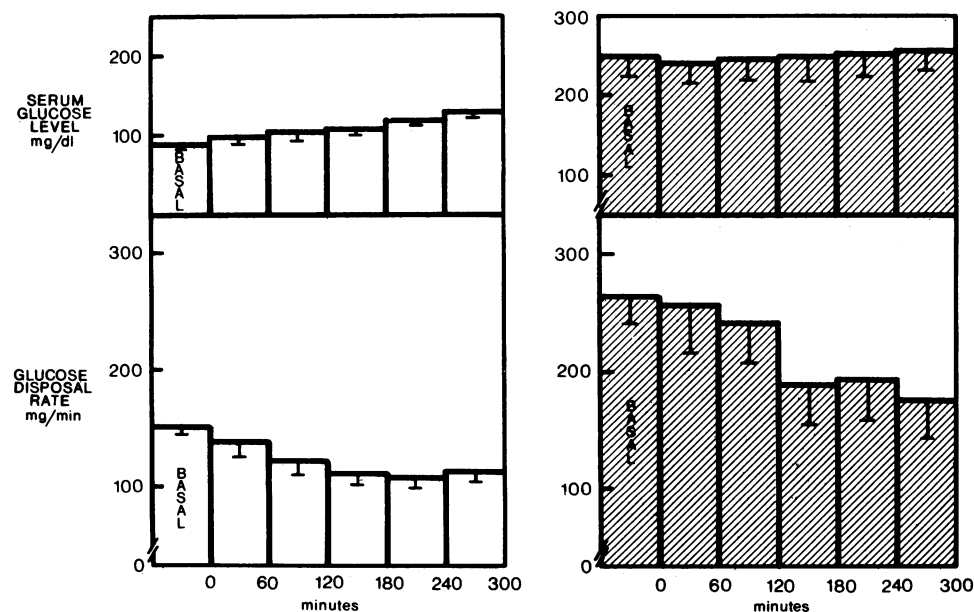Glucose clearance, via NIMGU mechanisms (NIMGU

Figure 1. Serum glucose levels (*top*) and rates of noninsulin-mediated glucose uptake in mg/min (*bottom*) during the infusion of SRIF with the serum glucose held at or near the fasting level in 11 control (open bars) and 7 NIDDM (hatched bars) subjects. Data are represented as mean±SEM.
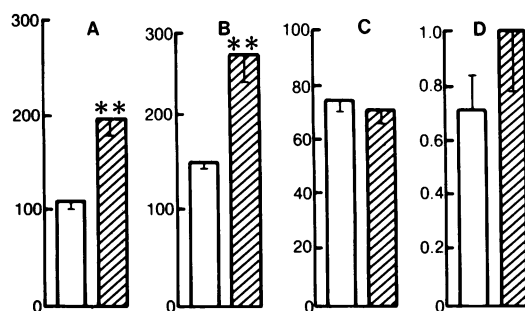
Figure 2. (A) Rates of NIMGU in mg/min at the basal serum glucose level; (B) basal rates of glucose disposal in mg/min; (C) rates of NIMGU as percent of the basal glucose disposal rate; and (D) rates of IMGU in mg/kg/min. Control subjects are in open bars, NIDDM subjects in hatched bars. *$P < 0.05$; **$P < 0.01$. Data are represented as mean±SEM.

÷ serum glucose level), calculated in control subjects decreased from 1.2±0.07 mg/dl per min at a serum glucose level of 90 mg/dl to 0.74±0.08 mg/dl per min at a prevailing serum glucose level of 248 mg/dl ($P < 0.01$). Since we have shown that absolute rates of NIMGU were similar in control and NIDDM subjects at a mean serum glucose level of 250 mg/dl, it follows that glucose clearance via NIMGU was similar in NIDDM and control subjects, 0.79±0.08 mg/dl per min compared with 0.74±0.08 mg/dl per min, respectively; $P =$ NS.

Discussion

Although a multitude of studies have assessed overall glucose uptake under hyperinsulinemic conditions (12, 13), only a few studies have examined rates of glucose uptake under conditions
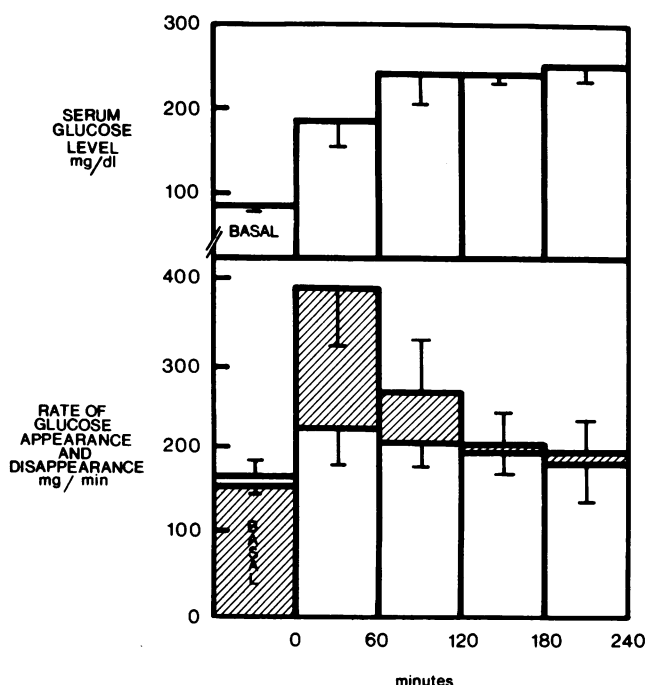

Figure 3. Rates of glucose appearance (hatched bars) and disappearance in mg/min (open bars) in seven control subjects during SRIF infusion with the glucose level clamped at ~250 mg/dl (top). Data are presented as mean±SEM.

of insulin deficiency. Gottesman et al. (2) reported that between 75 and 85% of glucose uptake in the postabsorptive state is non-insulin-dependent in normal human subjects. However, as previously described, these estimates were obtained through extrapolation and do not reflect a direct measurement. On the other hand, Felber et al. (1) used a methodological approach similar to ours, to look at the fate of glucose metabolism in normal humans, under insulinopenic conditions, and estimated that >80% of basal glucose uptake was independent of insulin action. However, in that study (1) NIMGU may have been over-estimated, since basal peripheral insulin levels were only suppressed by 50% to 7 μU/ml, during the SRIF infusion, and this degree of insulinemia may still stimulate glucose uptake. Furthermore, recent studies have shown that the t½ for deactivation of insulin action on in vivo glucose disposal (i.e., time required for glucose disposal rates to decrease by ½ after the discontinuation of an exogenous insulin infusion) is ~30–50 min (10). Since Felber et al. (1) used data obtained as early as 40 min after the onset of the SRIF infusion, it is possible that some residual IMGU from the presomatostatin insulin levels still existed.

In the present study, we have directly quantitated basal rates of glucose uptake under insulinopenic conditions, in control and Type II diabetic subjects. We found that 75% of basal glucose uptake is disposed of via NIMGU mechanisms in control, and 71% in NIDDM subjects. Since the absolute rate of basal R_d was markedly elevated in NIDDM (274±31 vs. 150±7 mg/min, respectively), it follows that the basal absolute rates of NIMGU were also markedly elevated in the NIDDM subjects (195±23 vs. 113±8 mg/min). However, the control subjects were studied under euglycemic conditions, whereas the NIDDM subjects were studied at hyperglycemia. Therefore, to determine the effects of hyperglycemia per se on NIMGU, the control subjects were also studied at glucose concentrations comparable to the NIDDM group (~250 mg/dl). Under these conditions, absolute rates of NIMGU (Fig. 4) were nearly identical, between the two groups. These lines of evidence strongly argue that the noninsulin-mediated uptake system functions normally in NIDDM. Since it is well known that NIDDM subjects demonstrate a marked impairment in overall insulin-stimulated glucose disposal (12), it would appear that the mechanisms responsible for decreased glucose transport in NIDDM are unique to those tissues with an insulin-responsive glucose uptake system.

The glucose transport system can be described by Michaelis-Menten kinetics, such that increases in glucose concentration will lead to progressively smaller increases in glucose uptake, until the transport system becomes saturated, at which point a further increase in glucose concentration will lead to no further increase in glucose transport. Several reports have described the saturability of the insulin-stimulated glucose uptake system in vivo in both normal and Type II diabetic subjects (14, 15), and in isolated human adipocytes (16). Gottesman et al. (2) have extrapolated that noninsulin-mediated glucose transport is also saturable. However, the only direct support for this notion comes from in vitro studies measuring glucose uptake in the absence of insulin. Though our results show that NIMGU rates do not increase proportionally with the ambient serum glucose level, these data are not sufficient to show that the entire NIMGU system is saturable. However, since the degree of fall in metabolic clearance rate is directly proportional to the fraction of total NIMGU that is saturated and thus fixed, the marked fall in glucose clearance from 90 to 250 mg/dl is consistent with the formulation that most of the saturation occurs below a prevailing
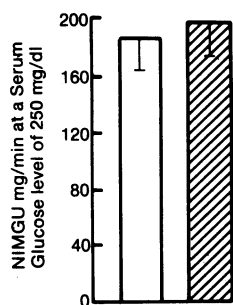
Figure 4. Rates of noninsulin-mediated glucose uptake (NIMGU) in mg/min, in seven control subjects (open bars) and seven NIDDM subjects (hatched bars) at a prevailing serum glucose level of ~250 ng/ml. Data are represented as mean±SEM.

serum glucose level of 90 mg/dl. Note that the NIMGU system as currently defined is not a homogeneous system, but rather represents a composite of multiple noninsulin-mediated glucose uptake systems likely to have different kinetic parameters in different tissues.

In the absence of insulin, glucose uptake can occur in both insulin-sensitive (muscle, adipocytes, etc.) and noninsulin-sensitive (nervous system, splanchnic, endothelial cells, white blood cells, red blood cells, etc.) tissues, and the current studies do not allow us to distinguish which tissues account for the measured rates of NIMGU. However, there is good evidence that the brain normally accounts for ~⅔ of basal glucose uptake (4, 17, 18), and since we have determined that ~⅔ of basal glucose uptake is noninsulin-mediated, it follows that the great majority of basal NIMGU in normal humans occurs in the CNS. This may not hold true for NIDDM subjects, where by mass action, the elevated basal glucose level may drive proportionally more glucose into insulin sensitive tissues than into noninsulin-sensitive tissues, via NIMGU mechanisms. Our studies do not directly demonstrate the tissue sites responsible for NIMGU, but it is likely that disposition of NIMGU differs in NIDDM compared with controls. For example, the major route of glucose metabolism in the CNS is oxidation (4, 17, 18), and it has been shown recently that basal rates of overall glucose oxidation are not increased in hyperglycemic NIDDM subjects (19). This is consistent with the formulation that NIMGU into CNS tissues is comparable in normal and NIDDM subjects, and that the increase in overall NIMGU in NIDDM involves increased glucose storage or other mechanisms of glucose disposal in non-CNS tissues. For example, in normal subjects at euglycemia, only a small component of overall NIMGU is into non-CNS tissues (including insulin target and non-target tissues). However, in the presence of hyperglycemia, the mass action effect of increased glucose levels may increase glucose uptake into these non-CNS tissues. This glucose either can be stored as glycogen or other macromolecules, or alternatively, can enter the glycolytic pathway and can be broken down to three carbon fragments, which, via the Cori Cycle and/or alanine cycle, are transported back to the liver where they can enter the gluconeogenic pathway; the glucose generated can then be stored as glycogen or can reenter the circulation. Under hormonal conditions that favor gluconeogenesis, i.e., insulin resistance and relative hyperglucagonemia found in Type II diabetics, this pathway could maintain a cycle of elevated rates of hepatic glucose production with increased rates of non-insulin-mediated glycolytic metabolism in peripheral tissues and recycling of the generated lactate, pyruvate, and possibly alanine back to the liver to sustain the elevated rates of gluconeogenesis and hepatic glucose output.

If hyperglycemia in NIDDM increases nonoxidative glucose metabolism in non-CNS tissues, while CNS oxidative glucose

metabolism remains normal, then since glucose oxidation is the major pathway of glucose metabolism in the CNS, it follows that the K_m for overall glucose uptake in the CNS must be substantially lower than that for non-CNS tissues (taken as a whole). Thus, due to relative saturation of CNS glucose uptake, increasing hyperglycemia will disproportionally augment glucose disposal into the non-CNS tissues. This would signify that if we could measure it, NIMGU into non-CNS tissues is increased in diabetics.

For any given serum glucose level, as one increases serum insulin levels, IMGU will increase; however, since NIDDM subjects are insulin-resistant, this increase in IMGU will be much greater in normal subjects compared with NIDDM. Our current studies show that absolute rates of IMGU are similar in both control and NIDDM subjects in the basal state. However, the determinants of the basal rate of IMGU are different in both groups. In NIDDM subjects normal rates of IMGU are achieved by means of a large "push" effect by the prevailing hyperglycemia (i.e., mass action) and by a small "pull" effect from basal insulin levels. In normal humans the reverse occurs, in that basal IMGU is a result of a relatively large insulin "pull" effect and small glucose "push" effect. This line of reasoning is consistent with a previous report from this laboratory, in which hyperglycemic clamp studies (20) were performed in Type II diabetic subjects at their respective fasting serum glucose levels over a range of insulin levels. These data showed that glucose disposal rates were superimposable on those obtained in control subjects clamped at euglycemia, over the same insulin concentrations. In view of these data, we suggested that the elevated basal rates of hepatic glucose output found in untreated Type II diabetics serve to maintain a prevailing level of hyperglycemia sufficient to compensate by mass action for the decrease in insulin effect to stimulate peripheral glucose disposal. In other words, hepatic and peripheral glucose metabolism are coupled in such a way that hepatic glucose output increases to the rate necessary to maintain the level of hyperglycemia required to compensate for the insulin resistance and thereby maintain IMGU at near normal rates. If this is the case, then for the insulin-sensitive uptake system to maintain "normal" uptake rates, the noninsulin-sensitive uptake system is subjected to high prevailing serum glucose levels with a marked increase in its rate of glucose disposal. In fact, we have found that basal rates of NIMGU in Type II diabetics were nearly twice those found in control, 195±23 mg/min vs. 113±8 mg/min, respectively. These findings may have implications in the pathogenesis of some of the late complications of Type II diabetes.

Most basal NIMGU normally occurs in noninsulin-sensitive tissues, particularly the CNS (4, 17, 18), and this has a critical impact on how one expresses rates of NIMGU. For example, since IMGU is known to occur principally in the muscle bed, IMGU rates should be normalized to some measure of the muscle mass or lean body weight (i.e., body weight, relative body weight, body mass index, etc.). On the other hand, in normal humans it would be more appropriate to express basal NIMGU rates normalized to brain weight or some other quantitative measure of the mass of tissue responsible for NIMGU. Since such measurements are unavailable, and since the tissues (mainly CNS) contributing to NIMGU are not proportional to body size, we have expressed NIMGU rates in total mg/min, as this avoids assigning a tissue site dependent on body size or weight. However, as discussed earlier, under hyperglycemic conditions, the mass action effect of glucose drives more glucose into NIMGU mech-

anisms, and this may occur in insulin-independent tissues (mostly CNS) and insulin-sensitive tissues (mostly muscle), or both. Logically then, the proportion of NIMGU going to muscle should be normalized to some aspect of lean body mass and that proportion of NIMGU going to the CNS expressed as total mg/min. Since we cannot measure the relative distribution of NIMGU into different tissues under hyperglycemic conditions, we expressed all rates of NIMGU in total milligrams per minute. Note, however, that regardless of the method of data expression, the conclusions we have drawn concerning comparative NIMGU rates between normals and NIDDMS, would be unaffected.

Finally, the validity of the data presented is critically dependent on certain assumptions that should be addressed: (a) SRIF-induced insulin deficiency must be as complete as possible. In this regard, serum insulin levels measured during the SRIF infusion were below the detectable limits of the assay and C-peptide suppression was nearly complete (>92%) in both groups when studied at their respective fasting serum glucose level and was >85% in the control group when studied at hyperglycemia. If we assume that hepatic C-peptide extraction is negligible (21), we can estimate the actual circulating peripheral insulin level. For example, fasting insulin levels were 9 μu/ml and 16 μu/ml in control and NIDDM subjects. Since C-peptide was suppressed by 92% in both groups this would result in actual peripheral insulin levels of <1 μu/ml and <2 μu/ml in control and NIDDM groups, respectively. Likewise, the control group studied at hyperglycemia would be expected to have had a circulating peripheral insulin level of <2 μu/ml. Therefore, it is highly unlikely that any IMGU existed during the SRIF infusions. (b) SRIF must not affect peripheral glucose uptake. This assumption seems reasonable, since several in vitro and in vivo reports have failed to show any effect of SRIF on glucose disposal at the doses used in this study (22). A recent report by Bergman et al. (23) suggests that SRIF may have an intrinsic effect on glucose uptake. Indeed, they showed a 65% increase in glucose clearance when SRIF was infused into dogs at a rate of 0.8 μg/kg per min. Note that this dose of SRIF is seven times greater on a per kilogram basis, than that employed in our study. Thus, it is unlikely that SRIF had any significant effect on glucose uptake under the study conditions used. (c) SRIF-induced growth hormone and glucagon deficiency must not affect peripheral glucose uptake. The available data indicate that glucagon has no effect on peripheral glucose uptake (24) and that short-term (<6 h) growth hormone (GH) deficiency does not alter basal glucose disposal (25). Finally, (d) NIMGU measured after 200 min of insulin deficiency must reflect that component of basal glucose uptake that is noninsulin-mediated. It is indeed possible that insulin deficiency induces changes in flux of certain metabolites, such as free fatty acids, amino acids, or other changes, which may affect glucose uptake, making it difficult to accurately assess the true basal physiologic NIMGU rate. We have good reason, however, to believe that our measurements accurately reflect basal physiologic NIMGU. We have estimated that NIMGU represents ~75% of overall basal glucose utilization in control subjects. These data are in good agreement with those of Gottesman et al. (2) who derived glucose uptake at "0" insulin by plotting glucose disposal vs. plasma insulin levels and extrapolated to the intercept for glucose disposal at "0" insulin. Though this method assumes linearity of glucose uptake over insulin levels lower than that employed in the study, this assumption is likely to be valid based on data from several in vitro studies (26, 27) showing glucose uptake to be linear over insulin concentrations ranging from 0 to 25 μu/

ml. In addition, numerous reports have estimated CNS (a non-insulin-sensitive tissue) glucose uptake to represent >70% of total basal glucose uptake (4, 17, 18). These estimates were derived using methods that did not alter the physiologic milieu. In addition, available data from Ferrannini et al. (28) and Thiebaud et al. (29) indicate that a two- to fourfold rise in the free fatty acid (FFA) level from basal is required to cause a ~20% decrease in insulin-stimulated glucose uptake in normal subjects. Felber et al. (1) have shown that the infusion of 600 μg/h of somatostatin (the dose used in this study) produced only a twofold rise in FFA levels in control subjects. Thus, it is unlikely that a rise in FFA levels significantly affected rates of noninsulin-mediated glucose uptake measured in our study. Recent reports (30, 31) have shown that the infusion of the amino acids leucine, isoleucine, and threonine to achieve high physiological serum concentrations significantly decreased forearm insulin-stimulated glucose uptake in humans. However, these same authors were not able to show any decrease in whole body glucose uptake during the infusion of each of these amino acids. This indicates that the decrease in forearm glucose uptake during amino acid infusion was very small when compared with overall glucose disposal, and therefore, could not be detected. Thus, changes in amino acid levels are not likely to have significantly altered our measurements of NIMGU. As with a rise in FFA levels, a rise in amino acid levels would have the net effect of decreasing glucose uptake. Therefore, although we do not believe that changes in FFA or amino acid levels have significantly affected our measurements of NIMGU, if indeed they had, then our measurements would represent underestimates of the true rates of NIMGU.

In summary, the results of this study document that the majority of basal glucose uptake occurs via noninsulin-mediated glucose uptake mechanisms. Furthermore, noninsulin-mediated glucose uptake seems to function normally in Type II diabetic subjects. Finally, Type II diabetics have basal rates of noninsulin-mediated glucose uptake that are approximately twice normal and this may play a role in the pathogenesis of the late complications of Type II diabetes.

Acknowledgments

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