Influence of Hyperglycemia on Insulin's In Vivo Effects in Type II Diabetes

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Abstract. The present study was designed to quantitate the interaction between the decrease in target tissue insulin action seen in subjects with Type II diabetes and the mass action effect of glucose exerted via the prevailing hyperglycemic state. To this end, euglycemic glucose clamp studies were performed in 26 control subjects using insulin infusion rates of 15, 40, 120, 240, and 1,200 mU/M^2 per min and in 10 Type II diabetic subjects using insulin infusion rates of 120 and 1,200 mU/M^2 per min. The results of these euglycemic studies indicated that insulin-stimulated peripheral glucose disposal was decreased in the Type II diabetics due to a combined receptor (rightward shift in the dose-response curve) and postreceptor defect in insulin action (decreased maximal response), whereas the decrease in insulin-mediated suppression of hepatic glucose output (HGO) was consistent with a defect in insulin binding (rightward shift in dose-response curve).

Hyperglycemic glucose clamp studies were also performed in the Type II diabetics at their respective fasting serum glucose levels (mean [±SE] 280±17 mg/dl) employing insulin infusion rates of 15, 40, 120, and 1,200 mU/M^2 per min. In the presence of their basal level of hyperglycemia, the noninsulin-dependent diabetes mellitus (NIDDM) subjects exhibited rates of overall glucose disposal that were similar to those observed in control subjects studied at euglycemia at similar steady state insulin concentrations. This suggests that in Type II diabetics, the mass action effect of glucose partially compensates for the marked decrease in insulin-stimulated glucose uptake observed under euglycemic conditions. However, even in the presence of hyperglycemia, insulin levels below 100 μU/ml had little effect and maximally effective insulin levels increased peripheral glucose disposal only 2.8-fold (142±7–413±47 mg/M^2 per min) above basal in the Type II diabetics, compared with a sixfold increase (75±4–419±34 mg/M^2 per min) in the control subjects studied at euglycemia. Thus, the severe insulin resistance that is a characteristic feature of NIDDM remains apparent.

Basal HGO was elevated in the NIDDM subjects (157±6 vs. 76±4 mg/M^2 per min for controls) and a high degree of correlation was found between the basal rate of HGO and the fasting glucose level ($r = 0.80$, $P < 0.01$). The presence of hyperglycemia augmented insulin-mediated suppression of HGO, but did not restore it to normal.

We conclude that: (*a*) in the presence of basal hyperglycemia, physiologic insulin levels exert a diminished effect to suppress HGO and stimulate peripheral glucose disposal in NIDDM; (*b*) basal HGO is elevated in untreated Type II diabetics, and this may serve to maintain the level of hyperglycemia required to compensate for the decrease in peripheral insulin action; and (*c*) fasting hyperglycemia exerts a suppressive effect on HGO but does not completely compensate for the decrease in hepatic insulin action in Type II diabetics.

Introduction

Insulin resistance is a prominent feature of the metabolic abnormalities seen in subjects with Type II or noninsulin-dependent diabetes mellitus (NIDDM)[1] (1–7). Recent studies from our laboratory, using the multiple euglycemic glucose clamp technique to assess the in vivo dose-response relationship for insulin action in subjects with Type II diabetes, indicated that

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1. *Abbreviations used in this paper:* HGO, hepatic glucose output; R_a, rate of glucose appearance; R_d, rate of overall glucose disappearance; NIDDM, noninsulin-dependent diabetes mellitus.

the peripheral insulin resistance results from a combined receptor and postreceptor defect (7). In subjects with mild fasting hyperglycemia (fasting serum glucose < 180 mg/dl), the insulin resistance was primarily due to a decrease in cellular insulin receptors (7). In contrast, patients with more severe fasting hyperglycemia (fasting serum glucose > 180 mg/dl) were more insulin resistant and exhibited a combined receptor and postreceptor defect in insulin action; the magnitude of the postreceptor defect was greater the higher the fasting glucose level. Thus, the postreceptor defect is the major abnormality in the subjects who are most insulin resistant (7). When the dose-response relationship for insulin-mediated suppression of hepatic glucose output (HGO) was examined in these subjects, a somewhat different picture evolved. Namely, the dose-response curve displayed a rightward shift in all subjects without a decrease in the maximal response, suggesting that the decrease in this biologic effect of insulin is due solely to the decrease in insulin binding (7).

These recent glucose clamp studies demonstrated that many Type II diabetics required steady state insulin levels of 300 μU/ml or greater to achieve euglycemia. Even at these insulin concentrations, the overall glucose disposal rate was only 50% greater than basal values compared with a sixfold increase above basal in normal subjects, and basal HGO was suppressed by only 75% (compared with 100% suppression in normals) (7). Since one would predict that lower insulin concentrations, such as those resulting from endogenous insulin secretion, would elicit even less stimulation of glucose disposal and suppression of HGO, these studies suggested that physiologic levels of insulin have little biological effect in Type II diabetics. Unfortunately, we were not able to test this hypothesis during the euglycemic studies because euglycemia could not be achieved within a reasonable period of time after the initiation of lower insulin infusion rates due to the marked insulin resistance.

In the present study, we have examined the dose-response relationship for in vivo insulin action in NIDDM subjects at their basal level of hyperglycemia. This approach allowed us to evaluate the effects of steady state serum insulin levels that were clearly within the physiologic range, to stimulate total glucose disposal rates, and inhibit HGO. Since fasting hyperglycemia increases glucose disposal by a mass action effect, we could also assess the relationship between each patient's fasting glucose level and their glucose disposal rates over a wide range of insulin concentrations.

Methods

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

Subjects. The study group consisted of 26 nonobese normal subjects and 10 nonobese subjects with Type II diabetes mellitus as defined by the criteria at the National Diabetes Data Group (8). The clinical and metabolic characteristics of the subjects are summarized in Table I. The serum glucose and insulin levels represent the mean of five determinations done on consecutive days.

The mean age (±SE) of the diabetic subjects was 52±3 yr compared with 39±2 yr for the normal subjects. The relative weights of the Type II diabetics ranged from 0.70–1.13, with a mean value of 0.93 for the control subjects (9).

After informed consent was obtained, all subjects were admitted to the University of Colorado Clinical Research Center and remained active to approximate their prehospital exercise level. Those subjects treated with insulin (patients 1, 3, 4, 5, and 6) or sulfonylurea agents (patients 2, 7, 8, 9, and 10) had these medications withdrawn for at least 3 wk before the study. All subjects were chemically euthyroid and no subject

Table I. Clinical and Metabolic Features

	Subject	Age	Sex	Relative weight	Fasting serum glucose	Fasting serum insulin
					(mg/dl)	*(μU/ml)*
	1	48	F	0.70	246	9
	2	54	F	0.94	372	5
	3	60	M	1.09	300	1
	4	56	F	1.13	363	9
	5	40	M	0.87	262	8
	6	45	M	1.13	235	19
	7	55	F	0.88	206	28
	8	50	M	0.99	276	21
	9	44	M	0.83	294	22
	10	68	M	1.10	249	30
Mean±SE		52±3	—	0.97±0.06	280±17	15±3
Controls	n = 26	39±2	17 F, 9 M	0.93±0.02	81±5	9±1

had a concurrent disease or was ingesting pharmacologic agents known to affect carbohydrate or insulin homeostasis.

Diet. All subjects were placed on a weight-maintenance (30 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 0800, 1,200, and 1,700 h, 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.

Euglycemic glucose clamp studies. In vivo insulin sensitivity was measured using a modification of the euglycemic glucose-clamp technique as previously described (7, 10–12). In normoglycemic subjects, the serum glucose was maintained between 80 and 90 mg/dl throughout the study period with a coefficient of variation of 3.5±0.5% by monitoring the glucose level at 5-min intervals and adjusting the infusion rate of a 20% dextrose solution. In subjects with fasting hyperglycemia, the serum glucose level was allowed to fall initially to euglycemic levels before the glucose infusion was initiated. After achieving euglycemia, the serum glucose level was maintained between 80 and 90 mg/dl and all studies were continued for an additional 80–220 min until the glucose infusion rate had been stable for 60 min.

The overall glucose disposal rate was assessed isotopically for each 20-min interval of the study (7, 13). Since it has been reported that glucose disposal rates increase during extended periods of hyperinsulinemia (14), only the last three 20-min intervals during which a true steady state existed were used in our calculations. These 20-min intervals were then averaged and the mean value used as the data point for the individual study. Urinary glucose losses were negligible under euglycemic conditions.

Each normal subject underwent three to five euglycemic glucose clamp studies on separate days. Insulin infusion rates of 15, 40, 120, 240, and 1,200 mU/M^2 per min were used to define the shape of the in vivo insulin dose-response curve. The number of separate subjects studied were 10, 21, 21, 11, and 11, at infusion rates of 15, 40, 120, 240, and 1,200 mU/M^2 per min, respectively. Six Type II diabetics were studied with an insulin infusion rate of 120 mU/M^2 per min, and all ten diabetics were studied with an insulin infusion rate of 1,200 mU/M^2 per min, in order to define their biological responsiveness to insulin under euglycemic conditions.

Hyperglycemic glucose clamp studies. In the diabetic group, each patient underwent glucose clamp studies with the serum glucose held at the fasting level (range 206–372 mg/dl). The serum glucose level was maintained constant by a variable rate glucose infusion (7, 12) with a coefficient of variation of 2.35±0.25%. Each of the 10 Type II patients received insulin infusions of 15, 40, 120, and 1,200 mU/M^2 per min for 140–220 min. Overall glucose disposal was also assessed isotopically during these studies (7, 13) and the glucose disposal rates were calculated for each of the last three 20-min intervals as described in the euglycemic studies. All studies were continued until the glucose infusion rate was stable for 60 min. The urinary excretion of glucose was measured during these studies and the glucose disposal rates were appropriately corrected.

Hepatic glucose output. The rate of glucose appearance (R_a) and the rate of overall glucose disappearance (R_d), were quantitated in the basal state and during each of the glucose clamp studies by infusing [3-^{3}H]glucose in a primed, continuous manner (7, 13). With this technique, 50 μCi of the tracer was injected as a bolus, followed by a continuous infusion at the rate of 0.50 μCi/min. Blood samples were obtained at 20-min intervals for the determination of both the concentration and specific activity of serum glucose. R_a and R_d were then calculated with the Steele equations (15) in their modified derivative form (13) since the tracer exhibits non-steady state kinetics under these conditions. The rate of HGO could then be calculated, since R_a represents the sum of HGO and the infusion rate of exogenous glucose. 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.

Analytical methods. Blood for serum glucose determination was drawn, placed in untreated polypropylene tubes, and spun using a Beckman 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 Beckman glucose analyzer (Beckman Instruments, Inc., Clinical Instruments Div., Fullerton, CA).

Blood for determination of serum insulin levels and serum glucose specific activity was collected in untreated tubes and allowed to clot. The specimens were then spun, and the serum removed and stored at −20°C until the determinations were made. Serum free and total insulin levels were measured by a double antibody radioimmunoassay (16).

Data analysis. All calculations were performed on a programmable calculator (Model 67, Hewlett-Packard Co., Palo Alto, CA). Data presented represent the mean (±SE) unless otherwise stated. Statistical analysis was done using the one-tailed t test for paired data and unpaired data as indicated. Correlation coefficients were calculated with the standard statistics package for the Model 67 calculator (Hewlett-Packard Co.).

Results

Clinical characteristics. The fasting serum glucose and insulin levels for all Type II diabetics are shown in Table I. All subjects were markedly hyperglycemic at the time of the study (mean glucose 280±17 mg/dl). Subjects 1–5 had fasting serum insulin levels that were in the normal range (<20 μU/ml) and had more striking fasting hyperglycemia than did subjects 6–10, who demonstrated fasting hyperinsulinemia (mean fasting serum glucose 309±26 vs. 252±15 mg/dl, $P < 0.05$).

Euglycemic glucose clamp studies. Both control and NIDDM subjects underwent euglycemic glucose clamp studies employing insulin infusion rates of 120 and 1,200 mU/M^2 per min. The mean glucose disposal rates of the NIDDM subjects were 162±26 and 193±30 mg/M^2 per min vs. 342±24 and 419±34 mg/M^2 per min in the control subjects at the insulin infusion rates of 120 and 1,200 mU/M^2 per min, respectively. Thus, the glucose disposal rates were markedly reduced in the diabetic subjects ($P < 0.001$) despite similar steady state serum insulin levels of 363±31 vs. 344±32 μU/ml, and 11,107±1,344 vs. 10,982±987 μU/ml, in the NIDDM and control subjects, respectively, at the insulin infusion rates of 120 and 1,200 mU/M^2 per min. These results indicate that the 10 NIDDM subjects were markedly insulin resistant with a large postreceptor defect in insulin action as evidenced by the 54% reduction in their glucose disposal rate at a maximally effective insulin concentration (~10,000 μU/ml).

In vivo dose response curves. Additional euglycemic glucose clamp studies were performed in each normal subject at insulin infusion rates of 15, 40, and 240 mU/M^2 per min. Each subject had at least three studies on different days, but it was not feasible to study every subject at all insulin concentrations. The sequence of insulin infusion rates was done randomly, except that the highest insulin infusion was always performed as the final study.

Hyperglycemic glucose clamp studies were done in each Type II diabetic at their fasting glucose level. This approach allowed a comparison of the dose-response curve at each individual's basal glucose level, euglycemia in controls and hyperglycemia in diabetics.

The individual hyperglycemic glucose clamp dose-response curves for the 10 Type II diabetics are shown in Fig. 1. The initial point on each curve represents the overall glucose disposal rate in the basal state measured isotopically and is equal to basal HGO minus urinary glucose loss. In the presence of the basal level of hyperglycemia, increasing steady state serum insulin levels led to a two- to sixfold increase in the overall glucose disposal rate in the Type II subjects.

The mean dose-response curves for insulin-stimulated glucose disposal for the normal subjects at euglycemia and patients with Type II diabetes at both hyperglycemia and euglycemia are shown in Fig. 2. These curves were constructed by estimating the glucose disposal rates from the individual dose-response curves at steady state serum insulin concentrations of 50, 100, 300, 1,000, and 10,000 μU/ml for the control subjects and at 50, 100, 300, and 10,000 μU/ml for the Type II diabetics. Inspection of the mean dose-response curves reveals a striking similarity between the glucose disposal rates for the Type II diabetics studied at hyperglycemia compared with normal subjects studied at euglycemia. In contrast, the data from the studies performed at euglycemia in the diabetic subjects (open circles) show a marked reduction in insulin-stimulated glucose disposal during both the 120 and 1,200 mU/M^2 per min insulin infusions. Thus, the basal level of hyperglycemia in the Type II subjects appears to largely compensate for the decreased peripheral insulin action, leading to rates of overall glucose disposal that approximate those observed in control subjects studied at euglycemia.

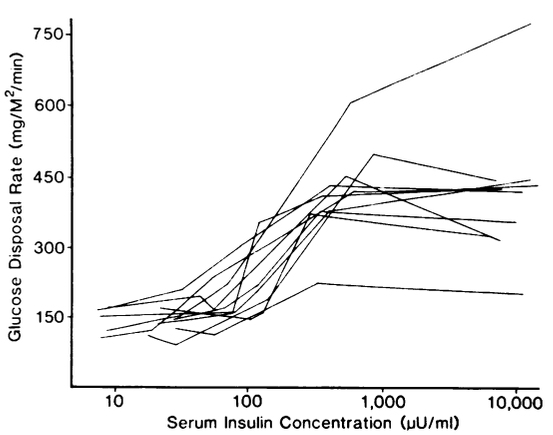

Figure 1. Individual dose-response curves for Type II diabetic subjects. Results were obtained by performing hyperglycemic clamp studies in each subject at insulin infusions of 15, 40, 120, and 1,200 mU/M^2 per min. The initial point on each curve represents glucose disposal in the basal state, as determined by the primed continuous infusion of [^{3}H]glucose (see text for details).

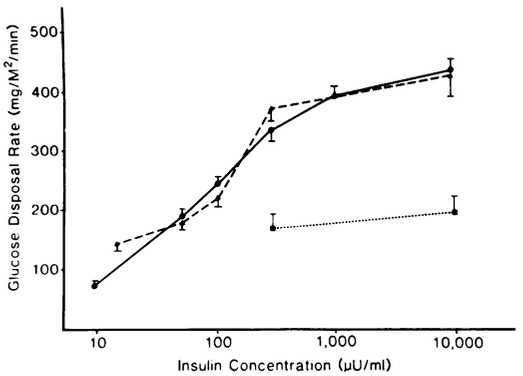

Figure 2. The effect of hyperglycemia on the dose-response relationship for insulin-stimulated peripheral glucose disposal in Type II diabetic subjects: normal subjects at euglycemia (●), Type II diabetics at hyperglycemia (▲), and Type II diabetics at euglycemia (■).

However, in the normal subjects at euglycemia, insulin led to a mean sixfold increase in overall glucose disposal rates with a ½ maximal insulin level of 90±14 μU/ml, whereas in the diabetic subjects studied at hyperglycemia, insulin only led to a 2.8-fold increase in glucose disposal rate with a ½ maximal insulin level of 150±30 μU/ml ($P < 0.025$).

Note that the measured rates of glucose uptake represent a cumulative total of uptake by both hepatic and peripheral tissues. While it is not possible to quantitate hepatic glucose uptake with the techniques employed, previous studies indicate that this uptake accounts for ~2–10% of overall glucose uptake under these conditions (17–19). Therefore, the preponderance of total glucose uptake is into nonhepatic tissues.

Hepatic glucose output. HGO was quantitated during each study by the administration of a primed, continuous infusion of [3-^{3}H]glucose. Basal rates of HGO were 76±4 and 157±6 mg/M^2 per min for the normal and Type II subjects, respectively ($P < 0.01$). In NIDDM the difference between basal rates of HGO (157±6 mg/M^2 per min) and basal glucose disposal (142±7 mg/M^2 per min, see Fig. 2) is attributed to the measured rate of renal glycosuria. As shown in Fig. 3, there was a high degree of correlation between the fasting glucose level and the basal rate of HGO in the Type II diabetics ($r = 0.80$, $P < 0.01$). Hence the diabetic subjects with the most severe fasting hyperglycemia had the highest rates of basal hepatic glucose production. A negative correlation was found between the fasting glucose level and the fasting insulin level as well as between the basal rate of HGO and the fasting serum insulin level, but neither of these relationships achieved statistical significance ($r = -0.60$, $P < 0.07$, and $r = -0.53$, $P < 0.12$, respectively). Multivariate analysis revealed that the relationship between the fasting serum glucose level and the basal rate of HGO remained significant when the fasting insulin level was held constant ($r = 0.68$, $P < 0.05$). However, when the rate of basal HGO was held constant, the relationship between the fasting serum glucose and insulin levels no longer approached significance, $r = -0.35$,

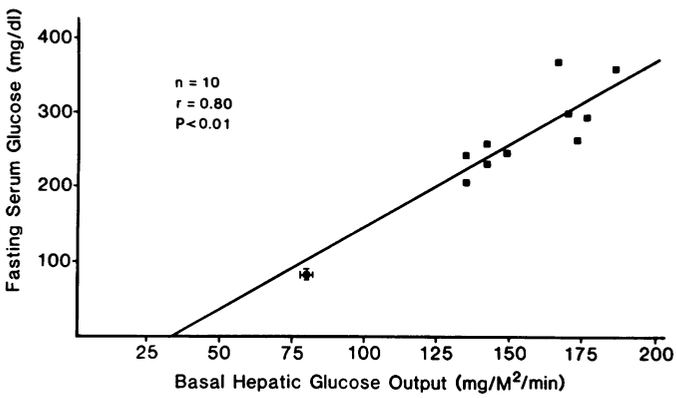

Figure 3. Correlation between fasting serum glucose and basal HGO in Type II diabetic subjects (■). The mean value for the control subjects (n = 26) (●) is plotted for reference. The regression analysis was performed using only the data for the diabetic subjects.

$P < 0.40$. Thus, this multivariate analysis supports the concept that the basal rate of HGO plays a significant role in determining the degree of fasting hyperglycemia in subjects with Type II diabetes.

The dose-response relationship for insulin-mediated suppression of HGO was examined by calculating the percent suppression of basal HGO at each insulin concentration. The mean dose-response curves were constructed by plotting the mean value for hepatic suppression as a function of the mean steady state serum insulin level for the respective infusion rate. The mean±SE steady state insulin levels were 34±2, 107±5, 329±20, 927±83, and 10,215±374 μU/ml for the normals, at insulin infusion rates of 15, 40, 120, 240, and 1,200 mU/M^2 per ml; for the diabetics studied at hyperglycemia the values were 44±6, 95±9, 416±35, and 10,669±942 μU/ml at insulin infusion rates of 15, 40, 120, and 1,200 mU/M^2 per min, respectively; and for the diabetics studied at euglycemia, the mean insulin values were 295±35 and 11,145±1,192 at insulin infusion rates of 120 and 1,200 mU/M^2 per min, respectively. As shown in Figure 4 *A*, the mean dose-response curve for the euglycemic studies in the Type II diabetics is markedly shifted to the right compared with the controls, although complete suppression was achieved at the highest insulin concentration in both groups. When the studies were performed under hyperglycemic conditions in the diabetic subjects, the dose-response curve was shifted leftward compared with the euglycemic studies. However, compared with normals, the dose-response curve was still right-shifted with a half-maximally effective insulin concentration of 42 μU/ml in the diabetic subjects studied at hyperglycemia, compared with a value of ~20 μU/ml for the controls. Thus, in the presence of hyperglycemia, insulin is more effective in suppressing HGO in NIDDM subjects. However, when compared with the control subjects, decreased sensitivity to insulin is still apparent, and this is manifested by the increased rates of basal hepatic glucose production in NIDDM and the rightward shift in the dose-response curve for insulin suppression of hepatic glucose production, even under hyperglycemic conditions. When the data are plotted in absolute terms (Fig. 4 *B*) it can be seen that the maximal decrements of hepatic glucose production are greater in NIDDM compared with normals. This follows from the findings that basal hepatic glucose production is greater in NIDDM, and that at maximal insulin levels suppression is 100% in both groups (Fig. 4 *A*). Nevertheless, the relative shapes of the dose-response curves are comparable, and the ½ maximal insulin levels are the same as those calculated from Fig. 4 *A*. Thus, the dose-response curve is right-shifted in the diabetics studied at hyperglycemia compared with controls (half-maximal insulin levels = 42 vs. ~20 μU/ml, respectively), but is less right-shifted than when the NIDDM subjects were studied at euglycemia.

Discussion

We have recently performed multiple euglycemic glucose clamp studies to evaluate in vivo insulin action in Type II diabetics (7). These studies suggested that the peripheral insulin resistance is due to combined receptor and postreceptor defects in insulin action, with the postreceptor defect being the predominant lesion in patients with fasting glucose levels >180 mg/dl (7). During these studies, insulin concentrations of over 300 μU/ml increased peripheral glucose uptake by only 50% above basal levels and suppressed HGO by only 75% (7). Since these insulin concentrations are several times greater than levels achieved by endogenous insulin secretion, these observations suggested the possibility that physiologic insulin levels may not have a major impact upon carbohydrate homeostasis in untreated Type II diabetics with significant fasting hyperglycemia.

In the present study, we have directly assessed this possibility by performing both euglycemic and hyperglycemic glucose clamp studies in 10 untreated Type II diabetics with marked fasting hyperglycemia. Using this approach, we were able to measure several aspects of in vivo insulin action in NIDDM subjects that could not be assessed during the euglycemic studies due to the relatively high insulin concentrations required to achieve euglycemia in face of the marked insulin resistance. Thus, in our earlier euglycemic dose-response studies (7), it was necessary to infuse relatively large amounts of insulin to bring the initial fasting glucose levels down to normoglycemic concentrations

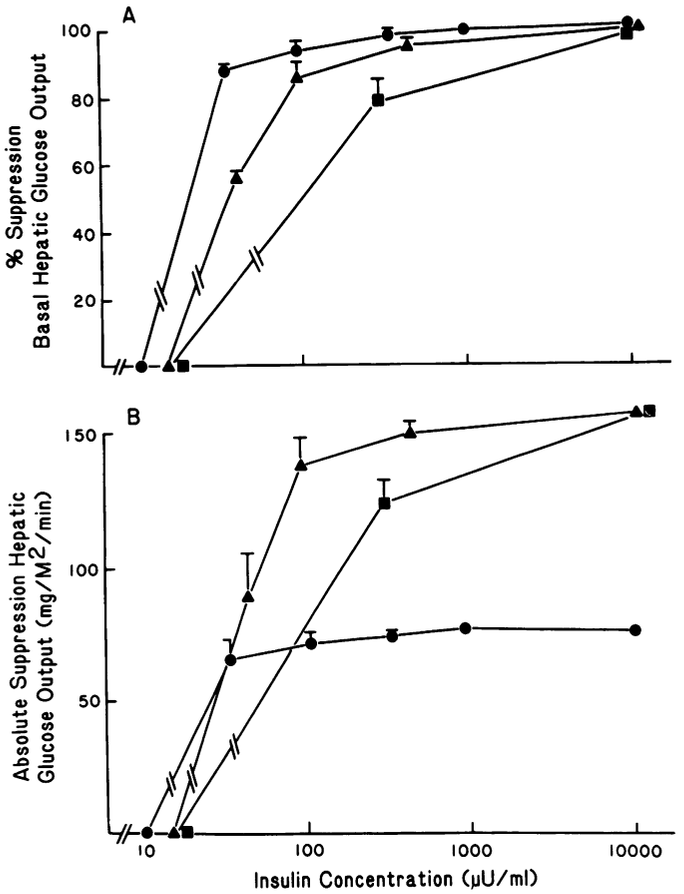

Figure 4. Insulin-mediated suppression of HGO: normal subjects at euglycemia (●), Type II diabetics at hyperglycemia (▲), and Type II diabetics at euglycemia (■). HGO was totally suppressed in all subjects at insulin concentrations of 1,000 and 10,000 μU/ml. Thus, no ±SE are shown for those points. (*A*) Percent suppression of HGO. (*B*) Absolute decrements in HGO at each insulin concentration.

in order to carry out euglycemic clamp studies. Because of the insulin resistance present in Type II diabetic patients, the lowest insulin level that was effective in achieving euglycemic glucose levels, in a reasonable period of time, was 300 μU/ml. In the present studies, we could assess the impact of physiologic insulin levels upon overall glucose disposal and suppression of HGO in the NIDDM subjects, since the hyperglycemic dose-response studies did not require any adjustment of the fasting glucose level before the clamp study.

To examine the effect of graded hyperinsulinemia upon overall glucose uptake in the presence of hyperglycemia, each of the diabetics was studied at insulin infusion rates of 15, 40, 120, and 1,200 mU/M^2 per min with the glucose level maintained at the fasting level. As shown in Fig. 1, insulin had a significant effect on the overall glucose disposal rate in the presence of the individual subject's basal level of hyperglycemia. However, when examined carefully, this apparent insulin effect diminishes in significance. From the mean dose-response curve for the hyperglycemic studies in the NIDDM subjects (Fig. 2), it is apparent that insulin concentrations <100 μU/ml have little impact upon overall glucose disposal. For example, raising the insulin concentration to 50 μU/ml (a greater than threefold increase over the mean basal level) led to only a 14% increase in glucose disposal. A similar increase in the insulin level in the control subjects led to a greater than twofold increase in overall glucose disposal. This is especially striking in light of earlier data showing that patients with Type II diabetes of this severity rarely achieve plasma insulin levels >50 μU/ml in response to regular meal ingestion (1, 20–22). Also, at maximal insulin concentrations, the glucose disposal rate increased only 2.8-fold above the basal value in NIDDM (142–413 mg/M^2 per min) compared with a sixfold increase in normal subjects (76–419 mg/M^2 per min). It should also be emphasized that the absolute glucose disposal rates for the control subjects would be much higher if the studies had been carried out in the presence of a similar degree of hyperglycemia. Thus, the normal subjects were clamped at glucose levels of 85 mg/100 ml, whereas the diabetic subjects were clamped at an average glucose level of 280 mg/100 per ml. The mass action effect of this 3.3-fold increase in glucose concentration to augment the rate of glucose disposal is considerable. For example, we have previously shown that when normal subjects are studied at a glucose level of 225 mg/100 per ml, the maximally insulin-stimulated glucose disposal rate is 698 mg/M^2 per min (23). It seems probable that these rates would be

even higher if normals were studied at a glucose level of 280 mg/100 per ml, which is the average value at which the glucose clamp studies were performed in the diabetic subjects.

As a result of the mass action effect of glucose (24–25), the prevailing hyperglycemia is responsible for achieving near normal absolute rates of glucose disposal during the dose-response studies and, based on the mean dose-response curves in Fig. 2, it appears that hyperglycemia plays an important role in compensating for the decrease in insulin action in the NIDDM subjects. Since each of the diabetics was studied at their respective fasting serum glucose levels, these results suggest that the degree of fasting hyperglycemia is determined in a relatively precise manner. In other words, the fasting glucose concentration appears to rise to the level required to adequately compensate for the decrease in insulin action in a given patient. The highly significant correlation between the fasting serum glucose level and the level of basal HGO, shown in Fig. 3 and demonstrated by others (27, 28), indicates a possible mechanism responsible for maintaining the elevated glucose level. Thus, we suggest that hepatic and peripheral glucose metabolism are tightly linked in such a way that HGO increases to the rate necessary to maintain the level of fasting hyperglycemia needed to compensate for the insulin resistance and maintain glucose uptake at near normal levels in insulin-dependent tissues. However, since our results represent static measurements made at one point in time, one cannot discern the true sequential course of events. Consequently, we cannot rule out alternative possibilities; i.e., that insulin resistance develops as a compensatory response to a primary increase in hepatic glucose production in order to restrain the rate of glucose uptake, and this, of course, would lead to hyperglycemia.

While it appears clear that glucose disposal and hepatic glucose production are closely linked, the factors responsible for maintaining the elevated rate of basal HGO in the NIDDM subjects remain to be identified. In normal man, hyperglycemia has been shown to exert a suppressive effect on HGO in the presence of a constant insulin level (29, 30). Thus, the elevated rate of HGO observed in NIDDM in the face of significant hyperglycemia appears contradictory. However, this could conceivably be another reflection of hepatic insulin resistance or could imply an abnormality in the mechanisms, whereby hyperglycemia suppresses hepatic glucose production in NIDDM. It is also possible that other factors are operative that stimulate HGO in NIDDM by increasing glycogenolysis and/or gluconeogenesis. For example, elevations in the levels of one or more of the counterregulatory hormones or increased hepatic sensitivity to these hormones could exist.

The mean dose-response curves for insulin-mediated suppression in HGO (Fig. 4) provide additional insight into another important aspect of hepatic glucose metabolism. It is apparent from these data that a similar decrease in insulin sensitivity has occurred in both the liver and peripheral tissues of the Type II diabetics. Furthermore, although a significant decrease in insulin's ability to inhibit hepatic glucose production exists in NIDDM under hyperglycemic conditions, the curve for suppression of hepatic glucose production is left-shifted in the presence of hyperglycemia compared with similar data generated in the NIDDM subjects under euglycemic conditions (Fig. 4). This demonstrates the additive effects of insulin and hyperglycemia in suppressing HGO in NIDDM. However, the presence of hyperglycemia does not completely compensate for the decrease in hepatic sensitivity to insulin, since hepatic glucose production is elevated in NIDDM at all physiologic concentrations of insulin. This point is best seen when the data for hepatic glucose production are presented in absolute terms (Fig. 4 *B*). With this method of data analysis, it can still be appreciated that when the diabetics are studied under euglycemic conditions there is a marked decrease in the sensitivity of the liver to insulin's suppressive effects on hepatic glucose production. Under hyperglycemic conditions, the decrease in sensitivity for this insulin effect is less, but the dose-response curve is still right-shifted compared with normal. Of further interest is the fact that at maximally effective insulin concentrations, the absolute decrements in hepatic glucose production are greater in the diabetic patients compared to the controls. This, of course, is because the basal rate of hepatic glucose production is elevated in the diabetic patients in the first place. The mechanistic interpretation of this is not clear; however, the elevation highlights the fact that at physiologic insulin concentrations, the absolute rates of hepatic glucose production are elevated in NIDDM subjects.

Decreased insulin receptors have been demonstrated in monocytes and adipocytes of NIDDM subjects (2, 7). If decreased receptors also exist in hepatocytes, then it is possible that this effect would contribute to the rightward shift in the dose-response curve (decreased insulin sensitivity) even in the face of hyperglycemia. It is also possible that the mechanisms whereby hyperglycemia suppresses HGO in normal subjects are altered in NIDDM patients, and represent a basic abnormality in intrahepatic intermediary metabolism in these subjects. Regardless of the mechanisms underlying this finding, these results highlight the importance of increased rates of hepatic glucose production in both the basal state and in the presence of elevated levels of insulin in maintaining the hyperglycemic, diabetic state.

Comparison of the effects of insulin to stimulate total glucose disposal (Fig. 2), with insulin's effects to inhibit hepatic glucose production (Fig. 4), demonstrate that the liver is more sensitive to insulin (at least insofar as suppression of glucose production is concerned) than peripheral tissues, for both control and diabetic subjects. This is reflected by half-maximally effective insulin levels for suppression of hepatic output of ~20 μU/ml for the control subjects and ~42 μU/ml for the diabetic subjects (under hyperglycemia conditions), compared with the respective half-maximal levels of 90 and 150 μU/ml for insulin-stimulated overall glucose disposal. Note, however, that this analysis slightly overestimates the degree of hepatic insulin sensitivity, since we have expressed suppression of hepatic glucose production as a function of the measured peripheral insulin levels. Since insulin in the portal circulation clearly contributes to suppression of

glucose production, and since a portal/peripheral insulin gradient exists, the integrated circulating insulin level to which the liver is exposed is somewhat higher than the peripheral insulin levels measured during the glucose clamp study. However, it must also be remembered that exogenous insulin suppresses endogenous insulin secretion. Therefore, during the infusion of exogenous insulin the portal/peripheral insulin gradient will narrow, and at the higher insulin infusion rates the unsuppressed endogenous insulin secretion would be small relative to the high rates of exogenous insulin infusion. Based on our own unpublished data, as well as published information from the literature (31), insulin secretion should be suppressed by at least 60–70% at all insulin infusions employed in the normal subjects. Thus, at the most, portal insulin levels might exceed the measured peripheral levels by ~10 μU/ml. This indicates that the apparent hepatic sensitivity to insulin is somewhat less than depicted in Fig. 4, but the sensitivity for this insulin effect would still be three times greater than that for insulin's ability to stimulate glucose disposal. Preliminary data (not shown) suggest that insulin's effect to suppress endogenous insulin secretion may be less in NIDDM subjects, and in this event the portal/peripheral gradient for insulin would be greater in the diabetics compared with the control. Insofar as this is the case, the data in Fig. 4 underestimate the degree of hepatic insulin resistance in NIDDM subjects, and the decreased sensitivity to insulin's suppressive effects on hepatic glucose production in NIDDM subjects may be somewhat greater than depicted in Fig. 4.

The potential impact of age upon these studies of peripheral insulin action must be considered. The diabetic subjects are significantly older than the control subjects (mean age 52±3 vs. 39±2 yr), and previous studies have documented the appearance of peripheral insulin resistance as part of the aging process (32–34). However, recent studies from our laboratory showed that the impact of aging upon peripheral insulin action does not become significant until the seventh decade of life (35). Therefore, it seems unlikely that the increased age of the diabetic subjects in the present study has a significant impact upon the interpretation of the results, since only one of these subjects was over the age of 60.

From the data generated in the present study, it appears that the prevailing level of hyperglycemia seen in an individual NIDDM patient largely compensates for the decrease in insulin action to stimulate glucose disposal. That is, the increase in glucose uptake due to the mass action effects of hyperglycemia (24–26) is about equal to the decrease in insulin-mediated glucose uptake due to insulin resistance. Since the mass action effect of hyperglycemia affects both insulin and noninsulin-mediated glucose uptake (26), noninsulin-mediated glucose uptake is also undoubtedly increased in the diabetic subjects. This can be best appreciated in the basal state. The basal glucose disposal rate in the Type II diabetics, assessed in the presence of their fasting serum glucose level, is elevated compared with the control subjects studied at euglycemia, 142±7 vs. 76±4 mg/M^2 per min. Since the absolute rate of insulin-mediated glucose uptake in the basal state is unlikely to be increased in the diabetic subjects, noninsulin-mediated glucose disposal must be significantly increased in these individuals. This increase in noninsulin-mediated uptake may be even greater postprandially, since the serum glucose level exceeds the fasting level throughout most of the day. Thus, glucose influx into insulin-independent tissues is undoubtedly increased in the Type II diabetic subjects and may play some role in the pathogenesis of the late complications of diabetes.

In summary, the results of the present study document the presence of markedly decreased insulin action in both hepatic and peripheral tissues in patients with Type II diabetes. At physiologic insulin levels, the hormone exerts a definite, but diminished, suppressive effect upon HGO in NIDDM. On the other hand, these insulin levels have little impact on overall glucose uptake. The presence of hyperglycemia, which is maintained by an increased rate of basal HGO, compensates for the decrease in insulin action via the mass effect of glucose to promote glucose uptake and inhibit hepatic glucose production. This suggests that peripheral and hepatic tissues are closely linked, such that the degree of hyperglycemia required to offset the decreased peripheral insulin effect prevails.

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