Locating the Site(s) of Insulin Resistance in Patients with Nonketotic Diabetes Mellitus

George Kimmerling, M.D., W. Curtis Javorski, M.D., Jerrold M. Olefsky, M.D., and Gerald M. Reaven, M.D., Palo Alto

SUMMARY

Insulin resistance and the ability of insulin to inhibit hepatic glucose production and to increase efficiency of glucose uptake were determined in 24 nonobese individuals: eight subjects with normal oral glucose tolerance, eight patients with chemical diabetes, and eight nonketotic patients with fasting hyperglycemia (> 150 mg. per cent). Insulin resistance was estimated by measuring the steady-state plasma glucose response to a continuous infusion of insulin, glucose, epinephrine, and propranolol. This approach permits us to inhibit endogenous insulin release, attain comparable steady-state plasma levels of exogenous insulin, and use the height of the steady-state plasma glucose response as a direct estimate of insulin resistance. The ability of insulin to inhibit hepatic glucose production and to increase efficiency of glucose uptake was calculated from the results of two studies in which a continuous infusion of ^{3}H-2-glucose was used to measure glucose turnover rate. The first study was performed after an overnight fast, under conditions of basal insulin levels, while the second was conducted during the infusion of insulin, glucose, epinephrine, and propranolol. Hepatic glucose production is equal to glucose turnover rate during the basal study and is equal to glucose turnover rate minus the infusion rate of cold glucose during the second study. Glucose uptake in both studies is equal to glucose turnover rate minus urinary glucose loss, and the efficiency of glucose uptake is calculated by dividing glucose uptake by the plasma glucose pool size. The mean (± S.E.) steady-state plasma glucose response was 113 ± 9 mg. per cent in normal subjects, 205 ± 14 mg. per cent in chemical diabetics, and 346 ± 30 mg. per cent in patients with fasting hyperglycemia. Thus, insulin resistance exists in nonketotic diabetes, and the greater the degree of glucose intolerance, the greater the insulin resistance. The resistance to the insulin infusion in patients with chemical diabetes seemed to be mainly a function of the inability of insulin to increase efficiency of glucose uptake, since insulin did retain its ability to inhibit glucose production (although not to normal levels). In contrast, the infusion of insulin neither inhibited hepatic glucose production nor increased efficiency of glucose uptake in patients with fasting hyperglycemia. Thus, the insulin resistance that exists in patients with nonketotic diabetes cannot be considered to be a global phenomenon. Significant differences exist in the responsiveness of various tissues to the two general aspects of insulin's action on glucose homeostasis, and these differences provide a physiologic basis for the variations in degree of over-all insulin resistance that are present in the three groups of subjects. DIABETES *25*:673-78, August, 1976.

Over the past 40 years a series of investigations have suggested that resistance to the action of insulin exists in patients with diabetes[1-9] and that insulin resistance may play a role in the pathogenesis of this syndrome. The insulin resistance has been described primarily in patients with nonketotic diabetes, and we have recently documented and attempted to quantify the degree of insulin resistance present in patients with chemical diabetes[10] and in nonketotic diabetics with fasting hyperglycemia.[11] However, although there is considerable evidence that insulin resistance exists in these diabetic patients, there is relatively little information as to the nature of the resistance to the action of insulin. Insulin can modulate glucose homeostasis by inhibiting hepatic glucose output and/or by promoting glucose uptake from plasma, and insulin resistance in nonketotic diabetes could result from an abnormal response to insulin of either or both of these processes. These two facets of insulin action can be

From the Department of Medicine, Stanford University School of Medicine, and Veterans Administration Hospital, Palo Alto, California.

Dr. Kimmerling was a Resident Clinical Associate, Veterans Administration (MRIS 4932). Dr. Javorski was a Resident Clinical Associate, Veterans Administration (MRIS 4585). Dr. Olefsky is a Clinical Investigator, Veterans Administration (MRIS 6488). Dr. Reaven is a Medical Investigator, Veterans Administration (MRIS 7363).

Address reprint requests to Gerald M. Reaven, M.D., Veterans Administration Hospital, 3801 Miranda Avenue, Palo Alto, Calif. 94304.

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separated and measured experimentally, and the present study was performed in order to determine the degree to which each of these aspects of insulin action is impaired in the insulin resistance of adult-onset diabetes. In order to accomplish this goal, we have determined over-all insulin resistance and the ability of insulin to both inhibit hepatic glucose output and increase efficiency of glucose uptake in normal subjects and in nonobese, nonketotic patients with either chemical diabetes or fasting hyperglycemia.

MATERIALS AND METHODS

Subjects

Twenty-four male subjects with either fasting hyperglycemia (plasma glucose > 150 mg. per cent), chemical diabetes, or normal carbohydrate tolerance were studied while hospitalized on the Stanford General Clinical Research Center. All patients consumed a weight-maintenance liquid-formula diet, consisting of 43 per cent carbohydrate, 42 per cent fat, and 15 per cent protein. The daily caloric intake of 35 Kcal/kg./day was divided into portions of 1/5, 2/5, and 2/5 served at 8 a.m., 12 noon, and 6 p.m. Daily weights were obtained on all subjects and were constant throughout the study period. Oral glucose tolerance tests were performed after three days of hospitalization on patients with fasting plasma glucose levels < 110 mg. per cent, using 40 gm. of glucose per square meter body surface area as the glucose challenge. Patients were considered to have chemical diabetes if the fasting plasma glucose value was < 110 mg. per cent, the one-hour plasma glucose value > 185 mg. per cent, and the two-hour value > 140 mg. per cent. Subjects were considered normal if their one-hour plasma glucose value was < 165 mg. per cent and their two-hour value < 130 mg. per cent. In this manner three experimental groups were obtained: eight normal subjects, eight chemical diabetics, and eight diabetics with fasting hyperglycemia (> 150 mg. per cent). Some pertinent clinical characteristics of the experimental subjects appear in table 1. All patients were in good general health, without evidence of hepatic or cardiac disease. None of the diabetic patients had received either insulin or oral agents, and ketonemia was not present.

Protocol

After one week of dietary stabilization, glucose turnover was determined after an overnight fast by the continuous infusion technic, utilizing ^{3}H-2-glucose. The infusion lasted for 150 minutes, and blood was withdrawn every five minutes during the last 30 minutes for determination of glucose-specific activity and measurement of plasma glucose and insulin levels. This was termed the basal study, and it permits calculation of hepatic glucose production and glucose uptake in the fasting state.

Glucose turnover was measured in a similar fashion one week later, only on this occasion insulin resistance was also determined. Thus, in addition to the continuous infusion of ^{3}H-2-glucose, patients also received a constant intravenous infusion of glucose (6 mg./kg./min.), insulin (80 mU./min.), epinephrine (6 μg./min.), and propranolol (0.08 mg./kg./min.) as described in several previous publications from our laboratory.[8,10,11] This will be termed the standard-infusion study. Under these experimental conditions, endogenous insulin secretion is inhibited and steady-state plasma glucose (SSPG) and exogenous insulin (SSPI) levels are reached by 90 minutes. As before, blood is drawn for determination of glucose-specific activity and plasma glucose and insulin levels every five minutes during the last 30 minutes of the 150-minute infusion period. This study permits us to determine the ability of identical amounts of insulin to limit the height of the SSPG response in various subjects (insulin resistance), the degree to which insulin suppresses hepatic glucose production, and the ability of insulin to increase efficiency of glucose uptake.

Analytic Methods

Blood for determination of plasma glucose, insulin, and tritiated glucose was drawn into test tubes containing EDTA. The plasma was quickly separated and aliquots stored at −20° C. Plasma glucose was measured by the glucose oxidase method, using a Beckman AutoAnalyzer (Beckman Instrument Corp., Tarrytown, N.Y.). Plasma insulin was measured by the method of Desbuquois and Aurbach.[12] The specific activity of tritiated glucose was measured by the method of Katz and Dunn.[13]

TABLE 1

Mean (± S.E.) age and relative weight of the experimental subjects

Group	Number	Age	Relative weight*
Normal	8	45 ± 4	.97 ± .02
Chemical diabetes	8	47 ± 4	1.01 ± .03
Fasting hyperglycemia	8	48 ± 4	1.02 ± .03

*As defined by Metropolitan Life Tables.

Calculations

A. *Hepatic glucose production (HGP).* During the basal study the liver is the only appreciable source of glucose, and under these conditions hepatic glucose production is equal to glucose turnover rate. Thus, HGP = glucose turnover (mg./min.) =

^{3}H-2-glucose infusion rate (cts./min.) ÷ specific activity of plasma glucose (cts./mg.)

However, during the standard-infusion study glucose turnover is determined while subjects are receiving a constant infusion of exogenous glucose (6 mg./kg./min.), and this must be subtracted from the total glucose turnover rate in order to obtain HGP. Thus, HGP = glucose turnover rate (mg./kg./min.)—infusion rate (6 mg./kg./min.).

By comparing HGP during the basal and standard infusion studies we can determine the ability of an exogenous insulin infusion (80 mU./min.) to suppress HGP.

B. *Efficiency of glucose uptake (E_G).* The net glucose uptake rate is determined by subtracting the amount (if any) of urinary glucose loss from the total glucose turnover. Once the glucose uptake rate is known, the efficiency with which this amount of glucose is removed from plasma can be estimated. Thus,

Efficiency of glucose uptake (E_G) = glucose uptake (mg./min.)—urinary glucose (mg./min.) ÷ plasma glucose pool (mg.)

where glucose pool = plasma glucose concentration × size of the plasma compartment (4.5 per cent of body weight in kilograms).

Comparison of E_G during the basal and standard infusion studies allows us to assess the ability of an infusion of exogenous insulin to promote glucose uptake in normal and diabetic subjects.

RESULTS

Figure 1 demonstrates that the over-all degree of insulin resistance, as measured by the height of the SSPG level during the standard infusion, is greater in the diabetic groups. Furthermore, the magnitude of this insulin resistance increases with the severity of the diabetic state, since the mean (± S.E.) SSPG levels were 113 ± 9 mg. per cent in normals, 205 ± 14 mg. per cent in chemical diabetics, and 346 ± 30 mg. per cent in the diabetic patients with fasting hyperglycemia. Figure 1 also indicates that the steady-state levels of exogenous insulin (SSPI) were comparable in all three groups, confirming the fact that we were assessing the ability of the different experimental groups to dispose of a glucose load under an identical insulin stimulus.

The ability of insulin to inhibit HGP is seen in figure 2. In normal subjects HGP suppressed from a basal rate of 3.0 ± 0.13 mg./kg./min. to 0.6 ± 0.18 mg./kg./min. during the standard infusion. This represents an 80 per cent decrease. In the patients with chemical diabetes HGP was 2.9 ± 0.16 mg./kg./min. in the basal state and fell to 1.3 ± 0.26 mg./kg./min. during the standard infusion. This represents a 55 per cent decrease in HGP, and while this is a highly significant difference ($p < 0.001$), the degree of suppression was less in chemical diabetics than in normals ($p < 0.05$). In patients with fasting hyperglycemia, basal HGP did not fall during the standard infusion, and thus, insulin had no suppressive effect in this group of patients.

The effect of insulin on efficiency of glucose uptake (E_G) in the three patient groups is depicted in figure 3. In normal subjects, E_G was 7.6 ± 0.3 per cent during the basal state and increased to 13.6 ± 1.5 per cent during the standard-infusion study. E_G went from 7.5 ± 0.5 per cent to 8.4 ± 0.8 per cent in patients with chemical diabetes and from 4.6 ± 0.5 per cent to 6.1 ± 0.6 per cent in patients with fasting hyperglycemia during the basal and standard-infusion studies. Thus, the ability of insulin to increase E_G

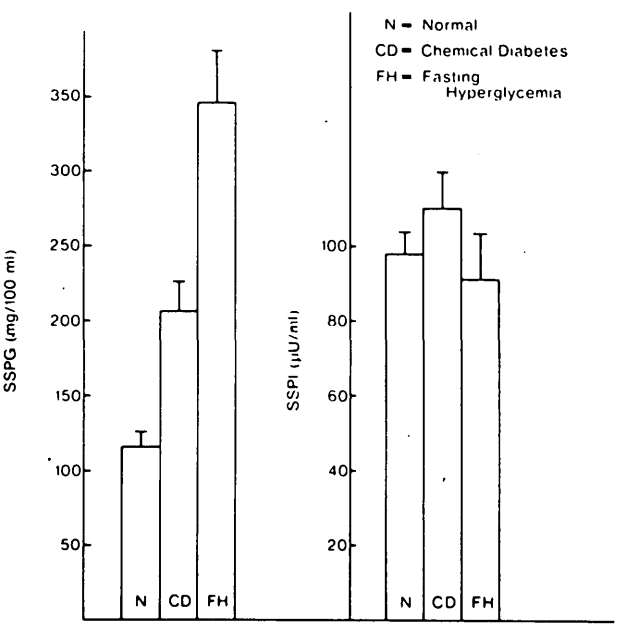

FIG. 1. Mean (± S.E.) steady-state plasma glucose (SSPG) and insulin (SSPI) responses to the infusion of glucose, insulin, epinephrine, and propranolol in normal subjects and in patients with either chemical diabetes or fasting hyperglycemia.

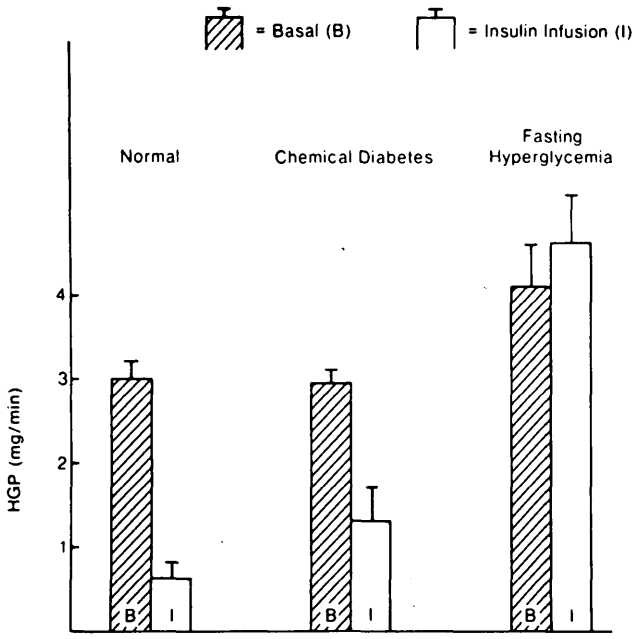

FIG. 2. Mean (± S.E.) values for hepatic glucose production (HGP) in the three groups of experimental subjects during the basal and standard-insulin-infusion studies.

was greatly, and essentially equally, attenuated in both groups of diabetic patients.

DISCUSSION

The results of these studies again indicate that patients with nonketotic diabetes are resistant to the action of insulin and as such confirm previous observations from many laboratories.[1-11] In this study we have attempted to define more precisely the locus of this insulin resistance by distinguishing between the ability of insulin to suppress hepatic glucose output and its ability to increase the efficiency of glucose uptake. The data we obtained demonstrate that differences exist in the relative responsiveness of these two facets of insulin's action, and that resistance to insulin-mediated glucose uptake seems to be the most basic lesion in patients with nonketotic diabetes. Thus, the ability of insulin to markedly increase efficiency of glucose uptake (E_G) during the insulin infusion in normal subjects was lost in both chemical diabetics and in patients with fasting hyperglycemia. On the other hand, insulin remained capable of significantly inhibiting hepatic glucose output (although not to normal levels) in patients with chemical diabetes, while it had essentially no effect on hepatic glucose production in diabetics with fasting hyperglycemia. These results indicate that insulin resistance cannot be considered to be a global phenomenon in patients with diabetes and that significant differences exist in the sensitivity of various tissues to the action of insulin in these patients. Furthermore, these differences in relative degree of sensitivity of target tissues to insulin provide a physiologic basis for the observed differences in the SSPG responses (insulin resistance) of the three groups of experimental subjects.

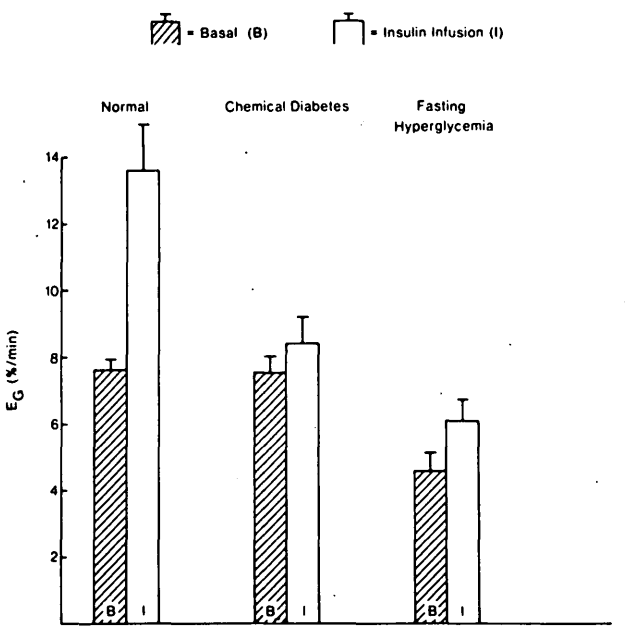

FIG. 3. Mean (± S.E.) values for efficiency of glucose uptake (EG) in the three groups of experimental subjects during the basal and standard-infusion studies.

It is somewhat difficult to put these results into perspective, as we are aware of no other study in which an attempt has been made to relate the degree of insulin resistance in diabetes to the ability of insulin to inhibit hepatic glucose output and promote glucose uptake. However, two groups of investigators, Kalant, Csorba, and Heller[14] and Forbath and Hetenyi[15] have used ^{14}C-glucose to separately estimate hepatic glucose production and glucose uptake in nonketotic diabetics with fasting hyperglycemia. The results of both studies are similar to ours in that the administration of either glucose and insulin[14] or glucose alone[15] was more effective in suppressing hepatic glucose production than it was in stimulating glucose uptake. Neither group investigated patients with chemical diabetes, but Forbath and Hetenyi[15] also studied ketotic diabetics and found them to be totally resistant to both facets of insulin's action. Thus, these authors also noted differences in the responsiveness of patients with one form of diabetes to the two different aspects of insulin's action, as well as observing that the ability of insulin to suppress hepatic glucose production seems to decrease with the severity of the

diabetes. Additional evidence for the notion that not all aspects of insulin's action are affected equally in diabetes can be found in the study of Issekutz and colleagues,[16] who demonstrated that insulin was more effective in suppressing hepatic glucose output than it was in promoting glucose uptake in dogs given alloxan or streptozotocin.

On the other hand, one basic difference exists between our results and those of Kalant et al.[14] and Forbath and Hetenyi.[15] Both of these groups indicated that hepatic glucose output could be suppressed in nonketotic diabetics with fasting hyperglycemia by the administration of insulin and/or glucose, whereas our results (in subjects that appeared similar to theirs) indicated that hepatic glucose production was not suppressed in such patients. Many variations in experimental design could account for this discrepancy, including differences in the patient populations, the previous treatment history of the patients, the isotope used (^{14}C-glucose vs. ^{3}H-glucose), the amount and route of administration of the insulin, the amount of nonisotopic glucose administered, and the inclusion in our insulin and glucose infusion of epinephrine and propranolol. Any of these variables might account for the observed differences in the ability of insulin to suppress hepatic glucose output in patients with fasting hyperglycemia, and it is impossible to decide at this time which results are "most correct." Indeed, the more important point seems to be that all of these studies, in spite of the very different experimental designs, came to essentially the same two basic conclusions. Specifically, resistance to the various actions of insulin is not present to an equal degree in a given diabetic subject, and resistance to insulin-mediated glucose uptake appears to be present in the mildest form of diabetes while resistance to hepatic glucose production seems to occur only as the severity of the diabetes increases. The significance of the fact that resistance to insulin-mediated glucose uptake is seen before resistance to hepatic glucose suppression develops remains to be clarified, but it seems reasonable to suggest that understanding the reason for this difference in insulin sensitivity will be of importance in defining the pathogenesis of nonketotic diabetes.

Finally, a few comments must be made about the nature of the isotope used to study glucose kinetics. The majority of previous studies have employed ^{14}C-glucose, and it seems quite clear that the use of this tracer will lead to an underestimation of total glucose turnover due to glucose recycling.[13,17] In this regard, it has been suggested that the use of ^{3}H-2-glucose, which loses the tritium in the hexose-isomerase reaction, will avoid the errors inherent with the use of ^{14}C-glucose and lead to a more accurate estimate of total glucose turnover.[13,17] On the other hand, there is a potential drawback with the use of ^{3}H-2-glucose, and that relates to "futile" glucose production. Thus, tritium in the 2 position can be lost as ^{3}H-2-glucose goes from glucose → glucose-6-phosphate → fructose-6-phosphate → glucose-6-phosphate → glucose. This would result in the apparent appearance of new unlabeled glucose, when in reality a futile cycle, producing no new glucose, has taken place. In this regard, we may have overestimated hepatic glucose production in the diabetic patients, thereby underestimating the ability of insulin to suppress hepatic glucose output, and we intend to repeat these observations using ^{3}H-3-glucose, which should minimize this potential error.[18] However, given all available evidence concerning the magnitude of such futile cycles,[17] it could alter our results only quantitatively, and the conclusion that insulin resistance is not global in patients with nonketotic diabetes would remain unchanged.

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