Defect in Glucose Removal in Nonketotic Diabetic Patients with Fasting Hyperglycemia

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SUMMARY

The present experiments were performed to determine the ability of insulin to stimulate glucose uptake in normal subjects as compared with nonobese patients with fasting hyperglycemia at similar steady state plasma glucose and insulin concentrations. The studies were carried out at two different steady state plasma glucose levels (approximately 250 and 350 mg/100 ml), and, in both instances, patients with fasting hyperglycemia removed approximately one-half as much glucose from the plasma as did normal subjects. Since the plasma insulin levels were comparable in the two groups (approximately 100 μU/ml), these results demonstrate that patients with fasting hyperglycemia are more insulin resistant than are normal subjects. Furthermore, the differences in glucose removal were observed under conditions in which the plasma glucose levels in the normal and the diabetic groups were similar; therefore, the insulin resistance of patients with fasting hyperglycemia is independent of differences in plasma glucose pool size. Finally, glucose removal increased proportionately in both experimental groups at the higher plasma glucose levels, suggesting that saturation of the glucose transport system does not occur at these plasma glucose concentrations. These results further document the fact that nonobese patients with significant fasting hyperglycemia are less responsive than normal subjects to the acute action of insulin that stimulates glucose removal from plasma. DIABETES 28:32–34, January 1979.

In previous articles we indicated that nonketotic diabetic patients with fasting hyperglycemia were more resistant to the action of insulin than were normal individuals[1,2] and that the resistance involved a loss of normal responsiveness to insulin-stimulated glucose uptake. However, there are alternative explanations for the experimental results reported. In the first place, insulin resistance was determined by comparing the height of the steady state plasma glucose (SSPG) concentration reached during a constant infusion of glucose and exogenous insulin, while endogenous insulin secretion was inhibited by a continuous infusion of epinephrine and propranolol. The SSPG levels were higher in diabetic patients than in normal subjects at a time when the steady state plasma insulin (SSPI) levels were similar, and this was interpreted as an indication that these patients were insulin resistant. On the other hand, the higher SSPG levels in patients with fasting hyperglycemia need not mean that these patients were more insulin resistant than normal but simply that their glucose transport system became saturated at the plasma glucose levels reached during the infusions. Secondly, these studies were carried out under conditions in which the glucose infusion rate and the insulin concentration were held constant, and the SSPG level, which was allowed to vary, served as a direct estimate of the degree of insulin resistance. Therefore, one cannot eliminate the possibility that the size of the plasma glucose pool independently influenced insulin sensitivity. Obviously, both of these considerations could modify the interpretation of our previously published results. Thus, the current experiments were undertaken to address these two issues.

METHODS

Patient population. Twenty-one adult male subjects (10 normal and 11 diabetic) were studied while hospitalized at the Stanford General Clinical Research Center. Their ages ranged from 32 to 59 yr, and their relative weights were between 0.8 and 1.2. All patients consumed a weight-maintenance liquid formula diet (35 kcal/kg/day) consisting of 43% carbohydrate, 42% fat, and 15% protein, divided into portions of 1/5, 2/5, and 2/5 and served at 8:00 a.m., 12:00 noon, and 6:00 p.m. Oral glucose tolerance tests were performed after 3 days of hospitalization, using 40 g of glucose per square meter of body surface area. Sub-

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jects were considered normal if their 1- and 2-h plasma glucose levels were less than or equal to 165 and 135 mg/dl, respectively. The designation of fasting hyperglycemia was used to define patients with a fasting plasma glucose level greater than 150 mg/dl. All patients were in good general health without evidence of hepatic or of cardiac disease, and none of the patients with fasting hyperglycemia were receiving either insulin or oral agents.

Experimental protocol. The standard experimental protocol consists of a continuous intravenous infusion of porcine insulin (80 mU/min), epinephrine (6 μg/min), propranolol (0.08 mg/min), and glucose. The solution was infused for 180 min via a Harvard pump (Harvard Apparatus Co., Millis, Massachusetts) into an antecubital vein. Blood samples were drawn from the opposite arm through a needle kept patent by a slow saline infusion. Under these experimental conditions, endogenous insulin secretion is suppressed and SSPG and SSPI levels are reached by 90 min and maintained for the remaining 90 min of the study.

Normal subjects and diabetic patients received two infusions, which differed only in the rate of glucose infusion. Diabetic patients were infused with 3 and 6 mg/kg/min, while normal subjects received 12 and 18 mg/kg/min.

In addition to the "cold" glucose, each infusate also contained [3-^{3}H]glucose. The addition of the radiolabeled glucose provided a means of measuring glucose turnover rate during the infusions. Glucose turnover rate was calculated from the following formula:

$$\text{Glucose turnover rate (mg/min)} = \frac{\text{[3-}^3\text{H]Glucose infusion rate (counts/min)}}{\text{Plasma glucose specific activity (counts/mg)}}$$

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 were stored at −20°C. Plasma glucose was measured by the glucose oxidase method, using a Beckman glucose analyzer (Beckman Instruments, Fullerton, California). Plasma insulin was measured by the method of Desbuquois and Aurbach.[3] The radioactivity of tritiated glucose was measured by the method of Katz and Dunn.[4]

RESULTS

To compare glucose uptake in normal and in diabetic subjects at similar SSPG and SSPI levels, 10 normal subjects were studied while they were receiving a glucose infusion of 12 mg/kg body weight per minute together with the other components of the standard infusion. This resulted in SSPG values comparable to those seen in four diabetic patients when they received 3 mg of glucose per kilogram of body weight per minute (Figure 1, left panel). The SSPI values are seen in the middle panel of Figure 1 and, if anything, were slightly higher in the normal subjects. This difference is most likely a reflection of our inability to totally suppress endogenous insulin secretion with epinephrine and propranolol at these high plasma glucose levels in these individuals. The lack of "breakthrough" in the diabetic subjects is almost certainly an indication of their basic state of insulinopenia. Measurements of glucose

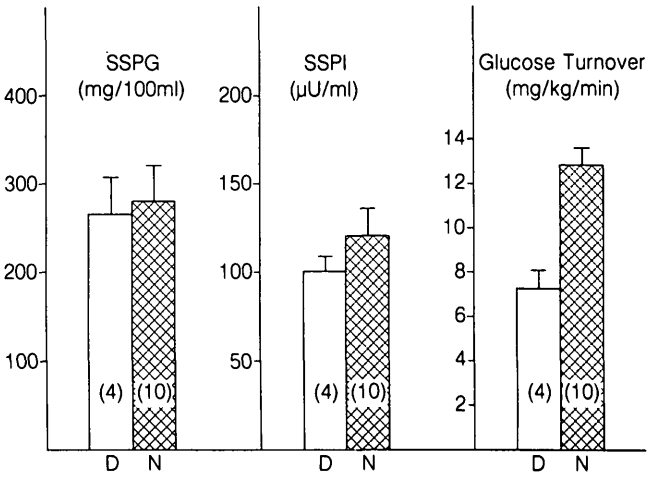
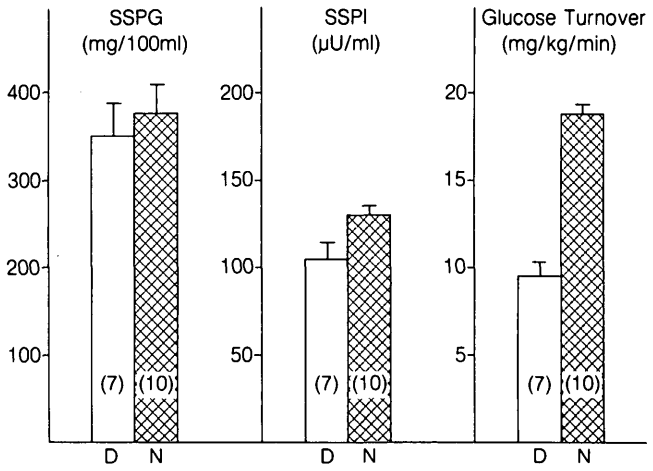


FIGURE 1. Mean (±SEM) steady state plasma glucose (SSPG) responses, steady state plasma insulin (SSPI) responses, and glucose turnover rates during continuous glucose infusion of 12 and 3 mg/kg/min, respectively, in 10 normal (N) subjects and four patients with fasting hyperglycemia (D).

turnover are seen in the right panel of Figure 1. Since the plasma glucose pool size was in a steady state during these studies, glucose turnover rate is equal to the total rate of removal of glucose from the plasma pool. These results demonstrate that the glucose turnover rate was 7.0 ± 0.8 mg/kg/min in diabetic patients as compared with 12.7 ± 0.7 mg/kg/min in normal subjects at comparable SSPG and SSPI levels. Thus, normal subjects removed approximately twice as much glucose from plasma than did patients with fasting hyperglycemia at similar hormone and substrate levels. It must be remembered that some of this glucose was lost in the urine. However, the glycosuria was similar in both groups and represented only a small fraction of the total removal of glucose from plasma (<5%). Thus, the figures for glucose turnover rate in Figure 1 closely approximate the whole body glucose uptake under these conditions.

Figure 2 presents data analogous to those seen in Figure 1, except that a glucose infusion rate of 18 mg/kg/

FIGURE 2. Mean (±SEM) steady state plasma glucose (SSPG) responses, steady state plasma insulin (SSPI) responses, and glucose turnover rates during continuous glucose infusion of 18 and 6 mg/kg/min, respectively, in 10 normal subjects (N) and seven patients with fasting hyperglycemia (D).



min was used in the 10 normal subjects to achieve SSPG and SSPI levels comparable with those obtained in seven diabetic patients who were given glucose infusions of 6 mg/kg/min. Glucose turnover (glucose uptake) was again measured by adding [3-^{3}H]glucose to the standard infusion mixture. The left panel of Figure 2 indicates that the SSPG levels attained under these conditions were similar in normal and in diabetic subjects. The SSPI levels (Figure 2, middle panel) were again slightly higher in the normal subjects. The determinations of glucose turnover in the two groups of subjects are seen in the right panel of Figure 2; they indicate that the amount of glucose removed from plasma in patients with fasting hyperglycemia was only 9.3 mg/kg body weight as compared with 18.3 mg/kg in normal subjects. Thus, the glucose removal rate of patients with fasting hyperglycemia was again much lower than that of normal subjects at comparable hormone and substrate levels.

Finally, it should be noted from the data in Figures 1 and 2 that the glucose turnover rate increased at the higher SSPG levels in both normal subjects and patients with fasting hyperglycemia. The fact that the glucose removal rate increased proportionately in the diabetic (33%) and normal subjects (44%) as the SSPG concentration increased demonstrates that the glucose transport mechanism had not been saturated in these patients at the lower SSPG levels.

DISCUSSION

In previous studies[1,2] we had indicated that patients with fasting hyperglycemia have a higher SSPG level than normal subjects during a constant infusion of epinephrine, propranolol, glucose, and exogenous insulin at a time when both SSPI levels and net glucose uptake were comparable in the two groups. These data were interpreted as evidence that patients with fasting hyperglycemia were more resistant than normal subjects to the ability of insulin to promote disposal of a glucose load. In this study we altered the experimental approach and equalized SSPG and SSPI levels, while letting net glucose uptake vary independently. When this was done (Figures 1 and 2), patients with fasting hyperglycemia removed significantly less glucose from plasma than did normal subjects, and these results provide additional evidence that these patients are insulin resistant. Furthermore, since glucose removal was decreased in diabetic patients at plasma glucose levels comparable to those of normal subjects, differences in plasma glucose pool size cannot account for the insulin resistance of diabetic patients seen in this and in previous studies.[1,2] On the other hand, one could argue that the observed differences between the two groups occur because the glucose uptake mechanism saturates at lower plasma glucose levels in patients with diabetes. However, the data presented in Figures 1 and 2 strongly suggest that this is not the case. Thus, in Figure 1 it can be seen that, at similar circulating levels of glucose and insulin, glucose uptake in patients with fasting hyperglycemia was only 7.0 mg/kg body weight per minute as compared with 12.3 mg/kg body weight per minute in normal subjects. When the SSPG level was increased, glucose uptake increased proportionately in both groups (Figure 2). These observations demonstrate that glucose removal mechanisms are no more saturated in diabetic patients than they are in normal subjects.

Although the data presented strongly support our previous contention that insulin resistance exists in patients with diabetes,[1,2] two qualifications must be made explicit. First, it should be pointed out that the combination of epinephrine and propranolol was employed in all infusions, and one cannot eliminate the possibility that under these conditions these agents exert a unique hyperglycemic effect in diabetics. We are not aware of any evidence for such an effect and, given the magnitude of the differences presented in Figures 1 and 2, this possibility seems highly unlikely. In this regard, it is worth noting that two reports have recently appeared in which conclusions essentially identical to ours have been reached after using methods that do not rely on epinephrine-propranolol suppression.[5,6] Second, our estimates of glucose turnover are dependent on certain assumptions, particularly that the concentration of radiolabeled glucose is constant during these measurements. More recently we have noted that this is not always the case when the plasma glucose pool size is expanded as the result of a decrease in the efficiency of glucose removal from plasma.[7] In this situation, glucose turnover will be overestimated. Consequently, we believe it possible that the absolute values for glucose turnover may be too high in the diabetic patients. In this event, glucose removal rates would be even less in the diabetic patients, and our results would have underestimated their degree of insulin resistance. Thus, neither of these issues would seem to negate the evidence presented, which indicates that patients with fasting hyperglycemia are insulin resistant as compared with normal subjects.

Finally, it should be noted that, in both groups of patients, glucose turnover rates were greater at the higher SSPG levels despite comparable plasma insulin levels. This indicates that increases in plasma glucose level over this concentration range can lead to substrate-induced augmentation of glucose uptake due to mass action effects.

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