Quantitation of Insulin-stimulated Glucose Disposal in Patients with Non-insulin-dependent Diabetes Mellitus

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SUMMARY

Glucose disposal rates (Rd) during an insulin clamp study reflect both basal and insulin-stimulated Rd. To quantify the amount of glucose taken up in response to a known increase in insulin concentration, two consecutive studies were performed on 10 patients with mild to moderate NIDDM (mean fasting glucose = 146 mg/dl) and 10 normal subjects. Endogenous insulin secretion was inhibited by somatostatin and plasma glucose level maintained at 180 mg/dl for 5 h. Rd (mg/m²/min) was determined isotopically for 2.5 h at insulin concentrations ~6 μU/ml and during 2.5 h of physiologic hyperinsulinemia at ~60 μU/ml (total glucose disposal), with the increase in Rd resulting from the approximate 10-fold elevation of plasma insulin concentration defined as insulin-stimulated glucose disposal. Results showed that the increment in Rd resulting from the elevation of plasma insulin concentration was relatively minor in NIDDM (38 ± 6), increasing from a mean (±SEM) value of 83 ± 8 to 121 ± 12. Similar values in normal subjects were 90 ± 7 and 274 ± 26 with an increment of 183 ± 21. Thus, insulin-stimulated glucose uptake in patients with NIDDM was only one-fifth of that in normals, and accounted for only 31% (38 ÷ 121) of total glucose disposal during the clamp study. These data indicate that the majority of previous insulin clamp studies of in vivo insulin action in patients with NIDDM, in which total glucose disposal and insulin-stimulated glucose disposal have been equated, have underestimated the magnitude of insulin resistance present in NIDDM. This conclusion is highlighted by the fact that the current study was carried out in patients with relatively mild NIDDM, and the possibility exists that physiologic increments of plasma insulin have essentially no effect on peripheral glucose disposal in patients with more severe NIDDM. DIABETES 1985; 34:831–35.

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Considerable evidence has accumulated in the past 15 yr[1-3] in support of the view that a decrease in the efficiency of insulin-stimulated glucose disposal characterizes patients with non-insulin-dependent diabetes mellitus (NIDDM). However, it is apparent that this conclusion is based to a considerable degree on experimental methods that compare estimates of glucose uptake in normal individuals and patients with NIDDM after the administration of exogenous insulin. Implicit in the approaches that have been used is the premise that basal glucose uptake, as distinguished from insulin-stimulated glucose uptake, is relatively minor in magnitude and comparable in all individuals. Since we were unaware of any experimental support for this crucial assumption, we thought it essential to initiate studies aimed at exploring this issue. To do so, we quantified glucose utilization rates in normal subjects and patients with NIDDM at both low insulin concentrations and after elevation of insulin concentration to values seen in plasma after meals. In this manner, we generated values for glucose utilization rates at low insulin concentrations, at physiologic levels of hyperinsulinemia, and, by difference, the increment in glucose uptake due to the increase in insulin level. The results of these measurements are reported below.

MATERIALS AND METHODS

Subjects. Ten patients with NIDDM and 10 normal individuals volunteered for this study. Their ages ranged from 42 to 72 yr and no subject had a body mass index (BMI) >30 kg/m².[4] Criteria for inclusion into the study were a diagnosis of normal glucose tolerance or NIDDM,[5] a fasting plasma glucose concentration <180 mg/dl, and good general health. No subject was receiving any medication known to affect carbohydrate metabolism. Specifically, none of the patients with NIDDM had ever been treated with insulin, and those taking sulfonylurea agents had their medication discontinued at least 4 weeks before admission. Some relevant characteristics of the two groups are seen in Table 1.

TABLE 1
Sex, age, body mass index (BMI), fasting plasma glucose (FPG), and insulin (FPI)

Group	M/F	Age (yr)	BMI (kg/m^2)	FPG (mg/dl)	FPI (μU/ml)
Normal	7/3	62 ± 2	25.9 ± 0.8	94 ± 2	9 ± 1
NIDDM	6/4	65 ± 2	27.1 ± 0.8	146 ± 8	11 ± 3

Estimation of insulin-stimulated glucose disposal. Insulin clamp studies were initiated after an overnight fast, and lasted for 5 h. During the first 150 min, insulin was infused at a rate of 2 mU/m^2/min, and then was increased to a rate of 25 mU/m^2/min during the last 150 min. Endogenous insulin secretion was inhibited by a constant somatostatin infusion of 250 μg/h. Blood samples were obtained every 5 min from an indwelling catheter in a hand vein, kept in a radiant warmer at 70°C to provide "arterialized" samples. Blood glucose was determined at bedside with a Beckman Glucose Analyzer II (Beckman Instruments, Palo Alto, California), and glucose infused at a rate needed to maintain plasma glucose levels at 180 mg/dl during the entire 300-min clamp period. Plasma insulin concentrations were measured by immunoassay.[6]

To quantify total glucose turnover, 60 μCi of ^{3}H-3-glucose was injected as an intravenous (i.v.) bolus at time zero and followed by a constant infusion of 0.32 μCi/min for the 5-h study. Aliquots of plasma were precipitated with $Ba(OH)_2$ and $ZnSO_4$ at 10-min intervals and plasma glucose concentration and radioactivity measured in the protein-free supernatant. Glucose specific activity was then determined, and the rate of disappearance of glucose (Rd) calculated using the non-steady-state equations of Steele.[7] Since these studies were performed at plasma glucose concentrations of 180 mg/dl, there was essentially no glucose excreted in the urine, and Rd provides a measure of total body glucose utilization. When endogenous glucose production is suppressed, as during the 25 mU/m^2 insulin infusion, the value of Rd is equivalent to the M-value determined by the insulin clamp technique.[8] Values for Rd were calculated for the 30-min periods between 120–150 min and 270–300 min, and used to estimate basal, total, and incremental (insulin-stimulated) glucose disposal. Endogenous glucose production was calculated as the difference between Rd and exogenous glucose infusion rate.

Statistical analysis. Data are presented as mean ± SEM, and Student's *t*-test was used to assess the statistical significance of the differences between the two groups.

RESULTS

Table 2 displays the plasma glucose and insulin concentrations observed during the infusions. These data demonstrate that we were able to achieve the desired steady-state plasma glucose level of 180 mg/dl in these studies. In addition, it is apparent that the steady-state plasma insulin levels were similar in both groups at both the lower and higher insulin concentrations.

Values for basal, total, and insulin-stimulated glucose uptake are seen in Figure 1. The results in the left panel indicate that basal glucose disposal rates were similar in the two groups. In contrast, total glucose uptake in patients with NIDDM was 44% ($P < 0.001$) of that of normal subjects (middle panel). Even more striking was the difference between the two groups in terms of insulin-stimulated glucose disposal, and in this instance patients with NIDDM had only 21% ($P < 0.001$) of normal values (right panel). Since the steady-state plasma insulin levels were comparable in both groups during these studies, the estimates of Rd provide an estimate of the ability of a given level of insulin to promote glucose disposal in normal subjects and patients with NIDDM.

Based on the data in Figure 1, it is apparent that the ability of added insulin to stimulate glucose disposal is markedly reduced in patients with NIDDM as compared with normal subjects. Specifically, increasing the plasma insulin level from 5 ± 1 to 57 ± 2 μU/ml in normal subjects (an increment of 52 ± 2 μU/ml) led to an approximate twofold increase in glucose disposal. To put these data in another perspective, 67% of total glucose disposal during the hyperinsulinemic clamp study in normal subjects could be attributed to the effect of the added insulin. In marked contrast, an equivalent increase in insulin level from 6 ± 1 to 59 ± 2 μU/ml (an increment of 53 ± 2 μU/ml) in the NIDDM group led to an approximate 0.5-fold increment in glucose disposal, and only 31% of total glucose disposal during the hyperinsulinemic clamp study resulted from the effect of the added insulin.

Total glucose disposal rates are conceptually similar to the M-value obtained during conventional insulin clamp studies, which is commonly used as an estimate of insulin-stimulated glucose uptake. If this had been the only measurement made, we would have concluded that glucose disposal was approximately 44% as great as normal in patients with NIDDM. On the other hand, if we compare the actual ability of insulin to promote glucose uptake a quantitatively different picture emerges, and insulin-stimulated glucose disposal was only 20% of normal in patients with NIDDM.

Although the above considerations document the fact that conventional insulin clamp studies significantly underestimate the magnitude of insulin resistance in NIDDM, they do not rule out the possibility that useful insights of a qualitative nature can be gained by this approach. To address this question, we defined the degree of correlation between total and insulin-stimulated glucose uptake. The results of this analysis documented an almost perfect linear correlation between the two variables ($r = 0.99$, $P < 0.001$) in all 20 patients. The relationship was present in both groups, but the degree was somewhat greater in the normal subjects ($r = 0.99$) than in patients with NIDDM ($r = 0.78$). On the other hand, the existence of this correlation does not negate the fact that the quantitative nature of the information gained will vary as a function of the approach used. As pointed out in the paragraph above, patients with NIDDM were approximately twice as "insulin resistant" when they were compared with normal on the basis of the ability of a known amount of insulin to stimulate glucose uptake.

TABLE 2
Mean (±SEM) steady-state plasma glucose (SSPG) and insulin (SSPI) concentrations during the infusion studies carried out at ~6 and 60 μU/ml of insulin

	SSPG (mg/dl)		SSPI (μU/ml)	
Group	~6 μU/ml	~60 μU/ml	~6 μU/ml	~60 μU/ml
Normal	181 ± 1	181 ± 2	5 ± 1	57 ± 2
NIDDM	183 ± 2	180 ± 1	6 ± 1	59 ± 2

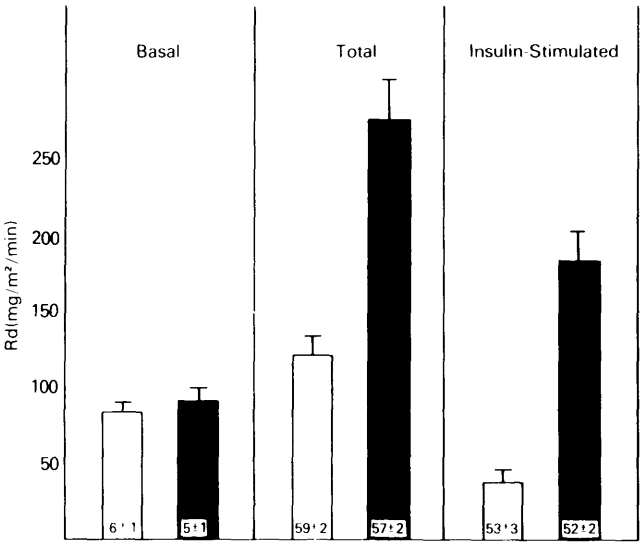

FIGURE 1. Mean (±SEM) values for basal, total, and insulin-stimulated glucose uptake in 10 normal subjects (■) and in 10 patients with NIDDM (□). The numbers in parentheses within each bar represent the mean (±SEM) insulin level at which the measurement of glucose was conducted.

Endogenous glucose production rates are seen in Table 3, and demonstrate that the values for this variable were similar in both groups at the lower and higher insulin infusion rates. Comparison of these data with the results shown in Figure 1 indicates that endogenous glucose production during studies carried out at the lower insulin level accounted for approximately 25% of the total Rd, and this was true of both groups. It is also apparent from these results that endogenous glucose production was abolished during the higher insulin infusion studies.

DISCUSSION

The present experiments were undertaken in an effort to differentiate between total and insulin-stimulated glucose disposal rates, and to quantify each of these values in normal subjects and in patients with relatively mild NIDDM. The results presented clearly indicated that the ratio of insulin-stimulated to total glucose uptake was quite different in the two groups. Specifically, insulin-stimulated glucose uptake contributed only 31% to total glucose uptake in patients with NIDDM, as compared with 67% in patients with normal glucose tolerance. In other words, only a minority of glucose taken up by patients with NIDDM during the period of physiologic hyperinsulinemia was actually due to the effect of the added insulin.

Although we have emphasized that the two-clamp method used in the current study provides a more accurate estimate of insulin-stimulated glucose uptake than does the conventional clamp approach of only estimating total glucose uptake, this should not be construed as an indictment of the utility of the clamp technique for determination of glucose uptake. For example, insulin-stimulated glucose disposal would also be underestimated by the insulin suppression test we have employed in the past.[9] Nor are we the first group to have estimated glucose uptake at different insulin levels in the same individual. On the other hand, we are unaware of any publication in which glucose uptake has been compared in normal subjects and patients with NIDDM, at the same plasma glucose concentration, and at both low and physiologic levels of insulin. The closest approximation we could find to the protocol we used was employed by Rizza et al.,[10] who assessed glucose uptake at plasma insulin concentrations approximating 50 and 100 μU/ml. Glucose disposal in patients with NIDDM was lower than that of control subjects at both insulin concentrations. However, the increase in glucose uptake when the insulin level was raised 50 μU/ml averaged 18 μmol · kg^{-1} in patients with NIDDM versus 21 μmol · kg^{-1} in normal subjects. Thus, they could not document any difference in the ability of insulin to stimulate glucose uptake in normal individuals as compared with patients with NIDDM when insulin was raised from 50 to 100 μU/ml. The reason for this apparent difference between their results and ours is not clear, but may relate to the fact that Rizza and colleagues did not quantify the ability of insulin to stimulate glucose uptake at both low and physiologic insulin levels. Another study that somewhat approximated our protocol was carried out by Revers et al.,[11] who compared glucose uptake in normal subjects and patients with NIDDM at insulin levels similar to those we used. However, their study was different from ours in that they studied normal subjects at plasma glucose levels of 80–90 mg/dl and in diabetic patients at plasma glucose levels between 206 and 372 mg/dl. In their report, they emphasized the fact that the insulin dose-response curves were "strikingly similar" when glucose disposal rates of patients with NIDDM were studied at hyperglycemia and normal subjects studied at euglycemia. On the other hand, inspection of their data indicates that glucose disposal rates at low insulin levels were elevated in patients with NIDDM, and glucose disposal increased to a greater degree in normal subjects when insulin was increased from about 10–100 μU/ml than in patients with NIDDM. Thus, despite equal values for total glucose disposal, the increment in glucose uptake due to insulin stimulation seemed reduced in patients with NIDDM. In this latter regard, and although their protocol was quite different from ours, the actual results are consistent with our findings. Finally, it should be realized that the same information could be achieved in a simpler manner by performing conventional clamp studies at each patient's fasting glucose concentration, determining both basal glucose uptake and total glucose uptake in response to physiologic hyperinsulinemia, and controlling for differences in fasting glucose level by expressing glucose disposal rate as glucose metabolic clearance rate. It is by far an easier approach, and one which we have encouraged.[12,13] Although we feel that this method can be supported,[12–15] other workers still question its validity,[16–18] and the resultant controversy has discouraged our use of this method. Consequently, we have turned to the protocol used in this study.

These results have two important implications concerning

TABLE 3
Mean (±SEM) endogenous glucose production rate (mg/m^2/min) during infusion studies carried out at ~6 and 60 μU/ml of insulin

Group	~6 μU/ml	~60 μU/ml
Normal	22.4 ± 8.7	0.5 ± 1.3
NIDDM	22.5 ± 8.3	2.4 ± 1.8

our understanding of the role of insulin resistance in the pathogenesis of NIDDM. In the first place, previous efforts to quantify insulin-stimulated glucose disposal in humans have primarily been based on the premise that glucose uptake during periods of physiologic hyperinsulinemia provides a reasonable estimate of insulin-stimulated glucose disposal. This was clearly not the case in the current study, in which two-thirds of glucose disposal observed in the NIDDM group during the period of hyperinsulinemia was unrelated to the effects of the added insulin. As a corollary, the degree of insulin resistance present in such patients with NIDDM has been underestimated in previous studies. To put it in perspective, raising the plasma insulin level from ~6 μU/ml to ~60 μU/ml led to an average increment in glucose disposal of 38 ± 6 mg/m²/min in patients with NIDDM as contrasted to an insulin-stimulated increment of 183 ± 21 mg/m²/min in subjects with normal glucose tolerance. In other words, insulin-stimulated glucose disposal in patients with NIDDM averaged only 20% of the value in normal subjects. On the other hand, these observations do not invalidate the qualitative insights gained by previous methods. For example, simply comparing total glucose uptake during the period of physiologic hyperinsulinemia in this study indicated that glucose disposal in patients with NIDDM was only 44% of that seen in normal subjects. Thus, resistance to glucose uptake was still seen in NIDDM when this approach was used, albeit at a much reduced level. Further evidence for the fact that measurements of total glucose uptake during conventional insulin clamp studies can provide useful information is the fact that an excellent correlation existed between total glucose uptake and insulin-stimulated glucose uptake in the 20 patients we studied.

In addition to documenting the fact that only a minority of total glucose disposal during physiologic hyperinsulinemia in NIDDM was in response to the increment in insulin, the results of the current study indicate that this phenomenon was true of patients with relatively minor degrees of fasting hyperglycemia. Thus, the mean (±SEM) fasting plasma glucose concentration was 146 ± 8 mg/dl for the entire group, and only five of the 10 subjects had fasting plasma glucose levels >140 mg/dl. These data emphasize the fact that resistance to insulin-stimulated glucose uptake is a prominent feature of even mild NIDDM, and indeed, this defect is already of major magnitude in patients with impaired glucose tolerance.[1,3] Since the patients with NIDDM we studied were extremely resistant to the ability of physiologic levels of insulin to stimulate peripheral glucose disposal, it seems likely that progression to severe fasting hyperglycemia takes place with relatively little further deterioration of this metabolic effect of insulin. Instead, it seems most reasonable to suggest that a defect in the ability of insulin to stimulate peripheral glucose disposal is necessary to develop impaired glucose tolerance and/or NIDDM, but that the development of severe fasting hyperglycemia is secondary to some other phenomena. For example, severe fasting hyperglycemia might only take place when patients with mild degrees of glucose intolerance are no longer able to sustain the degree of hyperinsulinemia that characterizes such individuals,[19-21] or when resistance to insulin-induced inhibition of endogenous glucose production occurs, or to a combination of these and other unidentified factors. In this latter regard, the current results strongly support the conclusion that the ability of insulin to suppress endogenous glucose production is preserved to a much greater degree in patients with mild NIDDM than is insulin-stimulated glucose uptake. Thus, endogenous glucose production was totally inhibited when insulin and glucose levels were raised in patients with NIDDM. On the other hand, the fact that endogenous glucose production was the same in both groups at either insulin level does not mean that the liver is absolutely normal in NIDDM, and differences might be found if a total insulin dose-response curve had been performed. However, the answer to this question is outside the scope of the present study, which was to try and provide a more quantitative approach to the measurement of insulin's ability to promote glucose disposal. As such, our results document the presence of severe resistance to insulin-stimulated peripheral glucose disposal present in patients with a relatively mild degree of NIDDM, and point out the apparent necessity to invoke some other metabolic defect(s) to account for the presence of severe fasting hyperglycemia in NIDDM.

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