Effect of Variations in Basal Plasma Glucose Concentration on Glucose Utilization (M) and Metabolic Clearance (MCR) Rates During Insulin Clamp Studies in Patients with Non-insulin-dependent Diabetes Mellitus

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SUMMARY

Two insulin clamp studies were performed at different steady-state plasma glucose concentrations in 13 patients with non-insulin-dependent diabetes mellitus (NIDDM). Steady-state plasma insulin concentrations were comparable, with mean ± SEM levels of 94 ± 3 and 95 ± 4 μU/ml being achieved during the two studies. Glucose utilization rate (M) varied directly with plasma glucose concentration in each subject. Thus, the mean ± SEM value of M was 4.92 ± 0.73 mg/kg/min when patients were studied at a mean ± SEM plasma glucose concentration of 226 ± 15 mg/dl, and M was 2.71 mg/kg/min when the same subjects were studied at a glucose concentration of 118 ± 6 mg/dl. In contrast, the values for glucose metabolic clearance rate (MCR), which were 2.35 ± 0.50 and 2.49 ± 0.47 ml/kg/min, respectively, during the two studies, did not vary significantly with plasma glucose concentration. These data indicate that the glucose metabolic clearance rate (MCR), but not glucose utilization rate (M), can be used to compare in vivo insulin action when insulin clamp studies are performed in subjects with different basal plasma glucose concentrations. DIABETES *31*:396–400, May 1982.

Although the insulin clamp technique[1] is an excellent way to assess in vivo insulin-stimulated glucose utilization, this approach as initially described has a major drawback when applied to patients with different basal glucose concentrations.[2,3] Quantification of insulin action with this technique is based on determination of the amount of glucose that must be infused (M) to maintain basal plasma glucose concentration during a period of sustained hyperinsulinemia. Since the rate of glucose utilization varies with plasma glucose concentration,[4] M can only provide an accurate estimate of in vivo insulin action when subjects are compared at the same ambient plasma glucose level. Obviously, this requirement drastically curtails the use of the standard insulin clamp to assess in vivo insulin action in patients with different fasting plasma glucose concentrations. One way to overcome this dilemma would be to relate the glucose disposal rate to the prevailing plasma glucose concentration, i.e., to calculate the metabolic clearance rate (MCR) of glucose. This approach has been used by Cherrington et al.,[4] who confirmed the fact that glucose utilization rate (M) increased when circulating glucose levels were increased in dogs. In contrast, glucose MCR was relatively constant over a wide range of plasma glucose concentrations. Attempts to apply this concept to normal man have met with mixed results.[5–7] Although we believe that these differences can be explained on the basis of variations in experimental design (vide infra), the issue is obviously of relatively minor importance in normal subjects with relatively small variations in basal glucose level. In contrast, the shortcomings of using M would be particularly relevant in comparing insulin action of normal subjects with that of patients with diabetes, and in diabetics before and after interventions aimed at lowering glucose. Therefore, we have carried out the current experiments in an effort to evaluate the relative utility of determining M, as compared with glucose MCR, in a series of patients with non-insulin-dependent diabetes mellitus (NIDDM) and varying degrees of fasting hyperglycemia.

MATERIALS AND METHODS

Patient population. Thirteen patients with NIDDM were admitted to the Stanford University Hospital General Clinical Research Center for these experiments. All subjects were volunteers, who gave informed consent for participation in the study. Ten patients were untreated, while three were receiving glipizide. Eight subjects were males, and five were females. Mean ± SEM fasting plasma glucose concentration on admission was 222 ± 14 mg/dl, with a range between 162–297 mg/dl.

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General protocol. The goal of the study was to compare the results of two insulin clamp studies, performed in each subject at two different steady-state plasma glucose concentrations. Each study was carried out for 3 h in the following general fashion. After an overnight fast, blood was drawn from an "arterialized" hand vein for determination of fasting plasma glucose concentration. A priming i.v. infusion of insulin (800 mU over 10 min) was followed by a constant infusion at 40 mU/m²/min. The glucose infusion was initiated 4 min after the insulin had been started (t + 4), at an empirically determined rated of 1–2 mg/kg/min. This was increased to 2.5–3.5 mg/kg/min at t + 10 min. Plasma glucose was determined every 5 min, and subsequent adjustments in the rate of glucose infusion needed to maintain the basal plasma glucose level were based on a negative feedback algorithm. The first servo-controlled adjustment was made at t + 12.5 min, when the plasma glucose concentrations at t + 10 min was known. The glucose infusion was continued for 180 min, and the amount of glucose metabolized (M) during the period 120–180 min, was calculated from the amount of glucose infused (I), with corrections made for changes in glucose pool size and urinary glucose excretion. M defines the amount of glucose that is metabolized during the period of hyperinsulinemia (at constant plasma glucose concentrations). As such, it provides a quantitative estimate of insulin action. To define the efficiency at which this glucose was metabolized, the metabolic clearance rate (MCR) of glucose was calculated by dividing the amount of glucose metabolized (M) by the plasma glucose concentration.

In one of the insulin clamp studies on each subject, plasma glucose was maintained at ±10% of the fasting value for 3 h by a 20% glucose solution given intravenously at a variable rate via an infusion pump (Harvard Apparatus, Millis, Massachusetts), adjusted every 5 min by a feedback algorithm to assure maintenance of basal plasma glucose level.[1]

In the other insulin clamp study, plasma glucose level was allowed to fall without glucose infusion for up to 120 min, or until a minimum plasma glucose of 80 mg/dl was achieved. The variable glucose infusion was then started and adjusted to maintain plasma glucose concentration at the new level, with variation of less than ± 10% for the third hour of the clamp study.

These clamp studies were performed in randomized order in each subject, an average of 7 days apart. The second insulin clamp for an individual subject was performed by different investigators, without knowledge of the results of the first study.

Determination of hepatic glucose production. Glucose turnover was determined in eight subjects, five untreated and three treated. A priming bolus of 62 μCi of 3-^{3}H-glucose was injected intravenously, followed by a constant intravenous infusion of 0.25 μCi of 3-^{3}H-glucose per minute for 6 −3 h prior to starting the clamp study and 3 h during the study. Plasma samples were obtained at regular intervals and analyzed for specific activity as previously described.[8] Rates of appearance and disappearance of unlabeled glucose during the clamp study were estimated by the non-steady-state equations of Steele.[9] Basal hepatic glucose production (basal HGP) was taken to be the rate of glucose appearance during the last half hour of tracer infusion prior to the start of insulin or glucose in the clamp protocol. HGP during the last hour of the insulin clamp was estimated by subtracting the rate of glucose infusion from the rate of glucose appearance calculated by the Steele equation.[9]

Analytical methods. Plasma glucose concentrations were determined by use of a modification of the glucose oxidase method,[10] and immunoreactive insulin by the method of Desbuquois and Aurbach.[11]

RESULTS

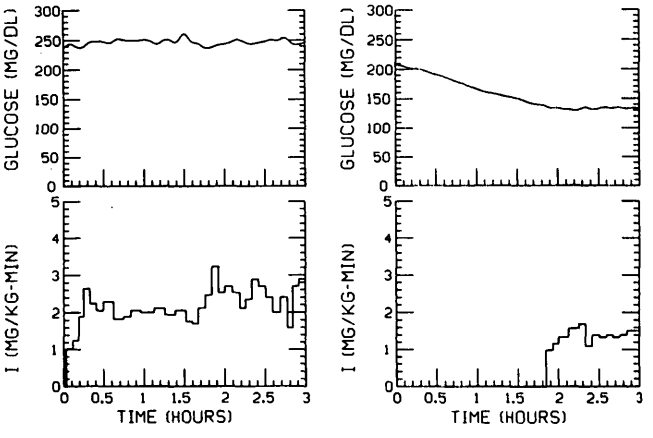
Figure 1 illustrates typical results of two insulin clamp studies performed in one patient. The left panel displays the steady-state plasma glucose concentration and glucose infusion rate observed during a 3-h period of hyperinsulinemia, with the plasma glucose concentration "clamped" at the basal glucose level. The right panel contains similar data obtained when the plasma glucose was allowed to fall during the first 110 min of the study, and then "clamped" at the lower level for the remainder of the 3-h period. It is obvious from this figure that considerably less glucose was infused when the patient was "clamped" at the lower glucose level (right panel).

The relationship between the amount of glucose metabolized (M) and the steady-state plasma glucose concentration during the third hour of the two insulin clamp studies for all 13 patients is depicted in Figure 2. Since the steady-state plasma insulin levels were the same in the two studies (94 ± 3 vs. 95 ± 4 μU/ml), it is obvious from these observations that the glucose utilization rate (M) increased directly as a function of the steady-state plasma glucose concentration.

In marked contrast are the data in Figure 3, which shows the relationship between the average glucose metabolic clearance rate (MCR) achieved during the third hour of the two clamp studies and the mean steady-state plasma glucose concentrations. It is apparent that there was no significant difference between the glucose MCR achieved during the two studies in each patient.

The data from Figures 2 and 3 are summarized in Figure 4. These results indicate that the average plasma glucose concentrations observed during the last hour of the two 3-h insulin clamp studies varied by approximately 100%. A sim-

FIGURE 1. Plasma glucose concentrations and glucose infusion rates during insulin clamp studies performed at basal (left panel) and reduced (right panel) plasma glucose levels in a patient with NIDDM. Plasma insulin levels were maintained at approximately 100 μU/ml for all 3 h of both sides.



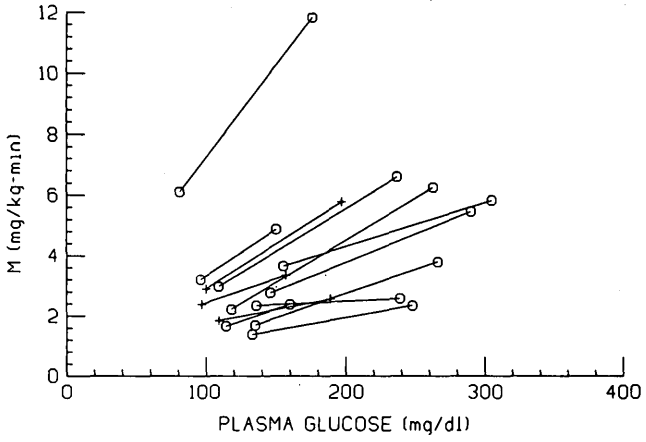


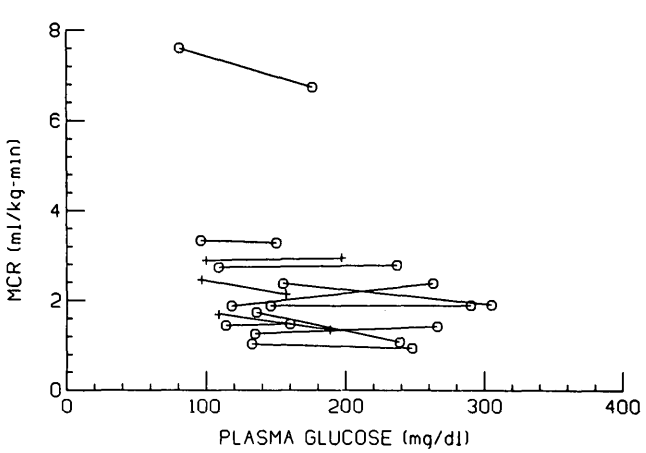
FIGURE 2. Relationship between glucose utilization rate (M) and plasma glucose concentration during the 3 h of the two clamp studies performed in 13 patients with NIDDM. 0, untreated patients; +, glipizide-treated patients.

ilar variation was seen in the determinations of glucose disposal rate (M). On the other hand, the glucose metabolic clearance rates (MCR) were the same in both studies. These comparisons are only valid if HGP was suppressed in both studies. The data in Table 1 suggest that this was the case. Thus, values for M rose significantly when patients were studied at the higher glucose concentration, but MCR remained essentially identical. Although these measurements were only made in 8 of the 13 subjects, it seems reasonable to assume that the results would have been similar in the remaining five patients.

DISCUSSION

The results of the present study (Figures 2 and 4) emphasize the crucial role that basal plasma glucose concentration plays in determination of glucose utilization rate (M) with the insulin clamp technique. It can be seen from these data that the value of M in 13 patients with NIDDM varied proportionately with the experimentally induced changes in plasma basal glucose concentration. Since there is no reason to believe that there had been any changes in the intrinsic ability of insulin to stimulate in vivo glucose utilization in these subjects, the experimental results unequivocally demonstrate that quantification of M with the insulin clamp technique cannot be used to compare insulin action in subjects with differences in basal plasma glucose concentration. As such, these results are very similar to those reported by Cherrington et al.[4] in the dog, and by Verdonk et al.[5] and Best and associates in man.[6]

FIGURE 3. Relationship between glucose metabolic clearance rate (MCR) and plasma glucose concentration during the 3 h of the two clamp studies performed in 13 patients with NIDDM. 0, untreated patients; +, glipizide-treated patients.



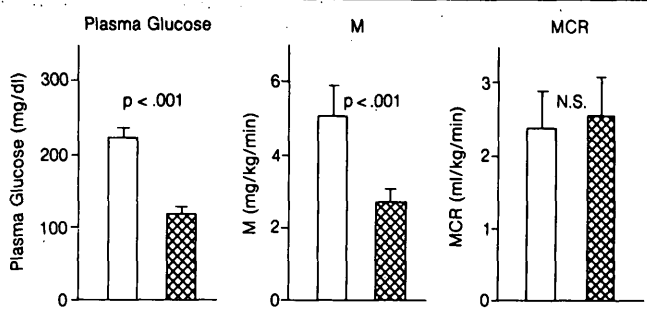


FIGURE 4. Mean + SEM values of plasma glucose, M, and MCR during the 3 h of the two clamp studies for all 13 patients with NIDDM.

However, the data in Figures 3 and 4 demonstrate that determination of glucose metabolic clearance rate (MCR), rather than M, provides a satisfactory method with which to compare the in vivo insulin action of individuals with different basal glucose concentrations. These results are in disagreement with the studies in normal man reported by Verdonk and associates[5] and Best and colleagues.[6] There are several possible explanations for this discrepancy. For example, it is conceivable that glucose utilization increases proportionately to plasma glucose level in patients with NIDDM, thus maintaining a stable MCR, but this does not happen in normal subjects. This formulation cannot be ruled out, but it appears to us to be an unlikely possibility.

Another hypothesis that must be considered is that our studies were experimentally flawed. It is possible that we overestimated glucose utilization (M) at the higher plasma glucose level due to artificially low values for urinary glucose loss. At the same time, we may have falsely reduced M during the studies at the lower plasma glucose concentrations by stimulating the release of various counterregulatory hormones. The net result would be a constant MCR. Although this possibility cannot be excluded, we think it an unlikely event for several reasons. In studies similar to ours, Santiago et al.[12] found that "significant increments in the mean absolute concentrations of epinephrine, norepinephrine, glucagon, GH, or cortisol were not associated with the 200 to 100 mg/dl decrement in the plasma glucose concentrations." However, they did note that increases of varying magnitude in all of these hormones were seen in some of the experimental subjects. Essentially similar results were reported by DeFronzo et al.,[13] who also noted great variation

TABLE 1
Values (mean ± SEM) of M and MCR when corrected for hepatic glucose production

Plasma glucose (mg/dl)	M		MCR	
	(mg/kg/min)	(mg/m²/min)	(ml/kg/min)	(ml/m²/min)
107 ± 8	2.88 ± 0.35	120 ± 19	2.76 ± 0.72	115 ± 27
232 ± 16	5.08 ± 1.14	214 ± 44	2.51 ± 0.34	105 ± 25

in both the number of patients who responded and the magnitude of the rise in counterregulatory hormone when plasma glucose was reduced in four diabetic subjects by a technique similar to the one we employed. Thus, there is no experimental support for the notion that our values for M at the lower glucose level were systematically reduced in an artifactual manner. Similarly, we do not believe that we overestimated M at the higher glucose level by systematically obtaining inadequate urine collections. Furthermore, it would seem unlikely that these two errors would cancel each other out in such a precise manner and thereby lead to a stable value for MCR.

A third and more likely possibility to us is that the difference between our results and those of Verdonk et al.[15] and Best et al.[6] is a function of the differences in experimental design. For MCR to remain stable, variations in plasma glucose concentration must be associated with parallel changes in glucose utilization rate. For this to occur, several conditions must be satisifed. In the first place, the studies must be conducted at plasma glucose concentrations at which saturation of the glucose uptake mechanism does not occur. Previous studies from our group have indicated that this is the case in normal subjects and patients with NIDDM up to plasma glucose levels of 450 mg/dl.[14,15]

Secondly, insulin levels must be high enough so that the majority of the total glucose that is being taken up is utilized by insulin-sensitive pathways. Under basal conditions, a considerable proportion of total glucose uptake is not dependent on insulin. If the plasma glucose level is increased, and insulin levels remain low, the increase in glucose utilization will be limited in magnitude. Theoretically, this is the situation in which glucose MCR is most likely to fall with elevations of plasma glucose concentration, and is precisely the experimental condition utilized by Verdonk et al.[5] Plasma insulin levels were maintained between 15 and 20 μU/ml in five normal volunteers by a somatostatin infusion. In this situation, glucose uptake is primarily of the non-insulin-stimulated variety; it does not increase proportionately as plasma glucose levels rise, and glucose MCR falls. These findings have been subsequently confirmed by a similar study of six normal volunteers by Best et al.[6] For example, in the latter study the fall in glucose MCR averaged 38% at a plasma insulin level of 1 μU/ml, and 16% at a plasma insulin level of 18 μU/ml, when the plasma glucose concentration was increased from approximately 90–180 mg/dl. However, as plasma insulin levels increase, the proportion of total glucose uptake that is non-insulin-stimulated falls, and the major portion of total glucose uptake becomes the insulin-stimulated fraction. Under these conditions, glucose disposal will more likely be proportional to plasma glucose level. This prediction seems to be confirmed by experimental data. Thus, in the study of Best et al.,[6] MCR only fell by 11% when plasma glucose levels were doubled at a steady-state plasma insulin concentration of 46 μU/ml, and this difference was not statistically significant. These data are almost identical to ours, in which we found an average fall in MCR of 9%, when patients with NIDDM were studied at insulin concentrations of approximately 100 μU/ml. This change in MCR was also not statistically significant, and comparable results have been reported in normal man in an abstract by DeFronzo and Ferrannini.[7]

In conclusion, there seems to be good evidence that determination of glucose utilization rate (M) with the insulin clamp technique cannot be used to compare in vivo insulin action of individuals with different basal plasma glucose levels. In contrast, and within the limitations outlined above, calculations of glucose metabolic clearance rate (MCR) appear to offer a means of carrying out the desired comparison. It is apparent that the rise in glucose utilization is not perfectly proportional to the rise in plasma glucose concentration, even at insulin levels of 100 μU/ml, and glucose MCR might fall by 10%. However, this variation is within the limits of the error of the technique,[2] and cannot even be shown to be statistically significant. Thus, it would appear that determination of glucose MCR provides a relatively good compromise in an effort to compare in vivo insulin action of individuals with different plasma glucose levels.

Alternatively, this problem can also be solved by studying all individuals at the same basal glucose levels. However, this approach is a technically tedious and cumbersome one. Furthermore, unless the clamp studies are carried out over the same time frame, there is the problem of constantly increasing values for M during prolonged insulin clamp studies.[3] Thus, in the absence of any contrary data, we would suggest that determination of glucose MCR, assuming an appropriate insulin level, provides a simple and reasonably accurate way to compare in vivo insulin action of subjects with different basal plasma glucose concentrations.

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