Relationship Between the Plasma Insulin Response to Oral Glucose and Insulin-stimulated Glucose Utilization in Normal Subjects

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SUMMARY

The relationship between in vivo insulin-stimulated glucose utilization (euglycemic clamp technique) and various estimates of the plasma insulin response to oral glucose was defined in 62 subjects with normal glucose tolerance. Both the incremental insulin increase above fasting ($r = 0.61$) and the total integrated insulin response ($r = 0.65$) were highly correlated ($P < 0.001$) with in vivo insulin action, and the relationship between total insulin response and insulin action remained significant ($r = 0.61$, $P < 0.001$) when corrected for variations in total glucose response, age, and obesity. In a subset of these subjects ($N = 27$) we were also able to assess state of habitual physical activity by estimating maximal oxygen consumption during bicycle ergometry. A significant correlation also existed between insulin action and response in these subjects ($r = 0.67$, $P < 0.001$), which remained significant ($r = 0.65$) when differences in total glucose response, obesity, age, and maximal oxygen consumption were taken into account. These data demonstrate that there is a significant correlation between insulin response and insulin action in normal individuals that can account for approximately one-third of the total variance in insulin action seen in subjects with normal glucose tolerance. Thus, determination of plasma insulin levels after an oral glucose challenge can only provide a qualitative estimate of insulin-stimulated glucose utilization. DIABETES *33*:460–463, May 1984.

The fact that the plasma insulin response to glucose can vary widely in normal subjects has been appreciated since the original paper by Yalow and Berson describing the immunoassay for insulin.[1] It is also clear that insulin-stimulated glucose utilization by normal subjects shows a comparable degree of variability.[2,3] Although these two observations need not be related, a consensus seems to have developed that is based on the assumption that elevated plasma insulin levels in subjects with normal glucose tolerance are secondary to a loss of normal insulin sensitivity, and that the hyperinsulinemia is a compensatory mechanism aimed at maintaining euglycemia. As a corollary, it has been generally accepted that the magnitude of an individual's insulin response to a glucose challenge is a reasonable reflection of that subject's insulin resistance. Although these two variables seem to be correlated in a general fashion,[3] we are unaware of any studies in which attempts have been made to quantify the degree of this relationship. In recent years, it has become obvious that factors other than insulin resistance can modulate plasma insulin levels. For example, variations in plasma insulin concentration could also result from differences in secretion of gastrointestinal hormones that modulate the beta cell response to glucose,[4] age-related changes in fractional catabolic rate of insulin,[5] and resistance to beta cell feedback inhibition by insulin.[6] Consequently, we felt it reasonable to measure both plasma insulin response to glucose and in vivo insulin action in a series of normal individuals, and to determine the degree to which the magnitude of the insulin response to an oral glucose challenge is a reflection of resistance to insulin-stimulated glucose utilization in this population.

MATERIALS AND METHODS

Subjects. The total study population comprised 62 individuals (group A), selected from a much larger pool of volunteers. Criteria for inclusion in the study were good general health, the lack of any disease or treatment known to affect glucose tolerance, fasting plasma glucose concentrations <110 mg/dl, an oral glucose tolerance test (OGTT) that excluded the diagnosis of diabetes mellitus or impaired glucose tolerance,[7] a negative family history of diabetes mellitus, and a body mass index [BMI = weight (kg) ÷ height

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(m^2)] less than 30 kg/m^2. A subset (group B) of these individuals, consisting of 14 males and 13 females, were also willing to undergo exercise testing. Comparison of the clinical characteristics of these two groups is presented in Table 1.

Experimental design. Volunteers were admitted to the Stanford General Clinical Research Center after 3 days on a diet that contained at least 200–250 g of carbohydrate. These diets were continued throughout the study period. Plasma glucose and insulin response during a standard OGTT were determined the morning after admission. Blood was drawn for the measurement of fasting plasma glucose and insulin concentrations at 0800 h after an overnight fast, the subjects were given a 75-g oral glucose load, and blood was drawn for repeated measurements of plasma glucose and insulin concentrations 30, 60, 120, and 180 min later.

In vivo insulin action was determined by the insulin clamp technique on the second morning after admission. Since this method has been previously described in detail,[8] only the general procedure will be outlined here. "Arterialized" venous blood samples were obtained from an indwelling catheter inserted retrograde into a hand vein placed in a radiant warmer maintained at 70°C. Plasma was immediately separated in a Beckman microfuge (Beckman Model S, Beckman Instruments, Fullerton, California), and glucose determined with a Beckman Glucose Analyzer II (Beckman Instruments). After establishing a baseline plasma glucose concentration, a primed continuous infusion of insulin (40 mU/m^2/min) was started. Plasma glucose was determined every 5 min thereafter. A variable infusion of glucose (200 mg/ml) was started 4 min after the start of the insulin infusion, and adjusted to maintain plasma glucose within 10% of the baseline value using a negative feedback algorithm. The amount of glucose metabolized (M) between 60 and 120 min of the study was computed from the amount of glucose infused, with corrections made for urinary glucose loss and changes in glucose pool volume. The validity of the glucose clamp technique to assess glucose utilization is based on the assumption that the amount of glucose infused to maintain basal glucose concentration is equivalent to the rate of glucose utilization. However, this is true only when hepatic glucose production is suppressed. To quantify the glucose utilization rate, total glucose turnover was determined during the clamp studies. This was achieved by a technique previously described from our laboratory,[8] in which [^{3}H]-3-glucose (62 μCi) was injected as an intravenous bolus 3 h before the start of the clamp study, followed by a constant infusion of 0.25 μCi/min until the clamp procedure was completed (5 h). Aliquots of plasma were collected at 20-min intervals, precipitated with $BaOH_2$ and $ZnSO_4$, centrifuged, and the protein-free supernatant evaporated in a scintillation vial. Plasma glucose concentrations and radioactivity were determined and glucose specific activity calculated.

The rate of appearance of glucose (Ra) and the rate of disappearance of glucose (Rd) were calculated at 20-min intervals before and during insulin infusion using the non-steady-state equation of Steele.[9] Ra and Rd, which should be equal during the period before the administration of insulin, yield the hepatic glucose output (HGO). Subtraction of the glucose infusion rate from the value of Ra, during the clamp study, yields HGO under the condition of hyperinsulinemia. Comparison of these two values defines the degree to which insulin has inhibited HGO. All measurements of M were corrected for residual HGO. For the purposes of data presentation, insulin resistance was defined as being equal to $^1/_M$.

Physical conditioning was estimated by determining maximal oxygen consumption ($M\dot{V}O_2$) during graded bicycle ergometry performed to exhaustion as described by Astrand and Rodahl.[10] $M\dot{V}O_2$ was defined as the peak value measured during the last 2 min of exercise testing.

TABLE 1
Clinical characteristics (mean ± SEM)

Variable	Group A (N = 62)	Group B (N = 27)
Age (yr)	42 ± 2	41 ± 3
Sex (M/F)	27/35	14/13
Fasting glucose (mg/dl)	88 ± 1	89 ± 1
Fasting insulin (μU/ml)	8 ± 1	7 ± 1
BMI (kg/m^2)	23.2 ± 0.4	23.7 ± 0.5

TABLE 2
Pearson simple correlation coefficients (r) between various estimates of insulin response and insulin resistance ($^1/_M$) in 62 normal weight, normal glucose tolerance individuals

Insulin	$^1/_M$
Insulin 0	0.27 ($P < 0.05$)
Insulin 30 min	0.29 ($P < 0.01$)
Insulin 60 min	0.53 ($P < 0.001$)
Insulin 120 min	0.49 ($P < 0.001$)
Insulin 180 min	0.51 ($P < 0.001$)
Incremental insulin	0.61 ($P < 0.001$)
Total insulin	0.65 ($P < 0.001$)

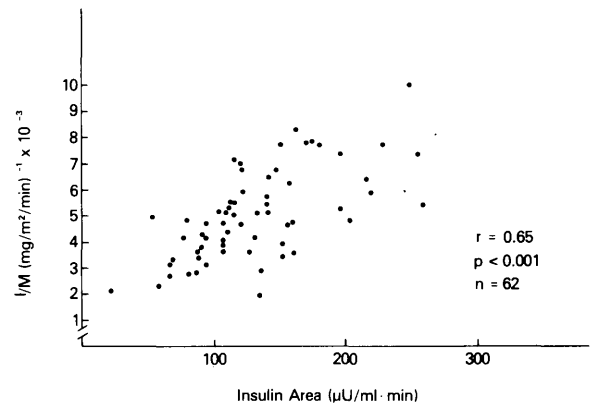

FIGURE 1. Relationship between the total integrated plasma insulin response to a 75-g oral glucose challenge and resistance to in vivo insulin-stimulated glucose utilization during euglycemic clamp studies ($^1/_M$) in 62 subjects with normal glucose tolerance.

TABLE 3
Pearson simple correlation coefficients between insulin resistance ($^1/_M$) and total glucose response, body mass index, and age

Variable	Correlation coefficient
Total glucose response	0.25
BMI	0.20
Age	0.09

Statistical analyses. Data are expressed as mean ± standard error of the mean (SEM). Pearson correlation coefficients, partial correlation coefficients, and stepwise multiple linear regression were performed using the Statistical Package for Social Sciences (SPSS).

RESULTS

Correlation coefficients (r) between insulin resistance ($^1/_M$) and various estimates of the plasma insulin response to oral glucose in the 62 subjects are seen in Table 2. These data indicate that the correlation between insulin resistance and plasma insulin level was statistically significant at every time point during the OGTT, as well as with both the calculated incremental and total integrated insulin response during the 3-h period. Mean (± SEM) steady-state plasma insulin concentration during these studies was 83 ± 2 μU/ml, and the correlations seen in Table 1 did not change appreciably when individual variations in steady-state insulin level were taken into consideration. Since the degree of correlation was greatest between total integrated insulin response and insulin resistance, all further analyses are based on use of this estimate of insulin secretion.

Individual data for all 62 subjects are seen in Figure 1, and these results confirm the presence of a general relationship ($r = 0.65$, $P < 0.001$) between the plasma insulin response to glucose and insulin action in this population of normal subjects. However, inspection of the individual data points serves to emphasize the fact that the insulin response to glucose can vary as much as fivefold in patients with comparable values for $^1/_M$. Thus, it is clear that factors other than variations in insulin resistance play a role in determining the plasma insulin response to glucose in any given individual.

Additional factors that have been reported to be associated with insulin resistance include oral glucose tolerance,[11] obesity,[12] and age.[13] Table 3 records the simple correlation coefficients noted between these metabolic variables and insulin resistance, and demonstrates that none of these shows any significant relationship to estimates of insulin resistance.

TABLE 4
Partial correlation coefficients between insulin response and insulin resistance ($^1/_M$) in 62 normal weight, normal glucose tolerant individuals, controlling for total glucose response, body mass index, and age

Controlled variable	r	r^2
—	0.65	0.42
Total glucose response	0.63	0.40
BMI	0.63	0.40
Age	0.65	0.42
Glucose + BMI + age	0.61	0.37

TABLE 5
Partial correlation coefficients for total insulin response vs. $^1/_M$ in 27 normal weight, normal glucose tolerant individuals controlling for total glucose response, body mass index, age, and maximum oxygen consumption

Controlled variable	r	r^2
—	0.67	0.45
1st Order		
Total glucose response	0.67	0.45
BMI	0.67	0.45
Age	0.67	0.45
$M\dot{V}O_2$	0.65	0.42
4th Order		
Total glucose, BMI, age, $M\dot{V}O_2$	0.65	0.42

On the other hand, variations in glucose tolerance, obesity, and age may have contributed to the relationship between insulin response and insulin resistance described in Figure 1. To evaluate this possibility, we computed the partial correlation coefficients between insulin response and insulin resistance, controlling for other factors thought to modulate insulin secretion. The results of this analysis are seen in Table 4, and demonstrate that the values for r and r^2 between insulin response and action do not change appreciably when differences in the total plasma glucose response during the OGTT, degree of obesity (BMI), or age are taken into account. Thus, the relationship between insulin response and action depicted in Figure 1 is independent of these additional variables. Finally, the r^2 value of 0.37 between insulin response and insulin resistance when all three additional variables are controlled for indicates that 37% of the variance between the individual insulin responses of these 62 normal subjects could be explained by differences in insulin action, independent of differences in glucose tolerance, obesity, and age.

If only 37% of the variance in insulin resistance can be accounted for by knowledge of changes in insulin response, what other variables might be playing a role in modulation of this important physiologic function? The only other environmental variable that we know affects in vivo insulin action in normal subjects is level of habitual physical activity as estimated by determination of maximal oxygen consumption.[12] Indeed, when we measured maximal oxygen consumption, as well as insulin action, in 27 of these subjects, a significant correlation ($r = -0.54$, $P < 0.01$) between these two variables was defined. There was also a significant correlation coefficient ($r = 0.67$, $P < 0.001$) between plasma insulin response and insulin resistance in these same 27 subjects. However, when we computed partial correlation

TABLE 6
Stepwise multiple linear regression analysis of $^1/_M$ in 27 normal weight, normal glucose tolerant individuals

Independent variable	Multiple r	r^2	P
Insulin response	0.67	0.45	< 0.001
$M\dot{V}O_2$	0.77	0.59	< 0.001
Total glucose response	0.78	0.61	NS
BMI	0.79	0.62	NS
Age	0.79	0.62	NS

coefficients in the 27 subjects (Table 5), it became clear that the correlation between insulin resistance and insulin response was independent of variations in maximal oxygen consumption.

Given the significant relationships between insulin resistance and both insulin response and maximal oxygen consumption, it seemed of interest to consider whether both of these variables might increase our ability to account for the variance in insulin resistance seen in these normal subjects. To evaluate this possibility, we carried out stepwise linear regression in the 27 subjects in which all variables had been measured. These data are seen in Table 6, and indicate that total insulin response alone accounted for approximately 45% of the variance in insulin action in this group of 27 subjects. It also can be seen that adding measurements of habitual physical activity (maximal oxygen consumption) to the analysis significantly improved ($P < 0.001$) the degree of correlation, and insulin response *plus* maximal oxygen consumption accounted for 59% of the variance in insulin resistance in these normal subjects. Finally, the data in Table 6 again demonstrated that glucose response, obesity (BMI), and age made no significant independent contribution to variance in insulin action in normal subjects.

DISCUSSION

The results of the current experiments can be viewed in several ways. At the simplest level, they demonstrate that there is a significant relationship in normal subjects between the plasma insulin response to an oral glucose challenge and insulin-stimulated glucose utilization as measured by the euglycemic clamp. This relationship was seen despite the putative confounding effects on the plasma insulin response of variables such as differences in the secretion of gastrointestinal hormones, catabolic rate of insulin, age, obesity, etc. Indeed, the fact that this relationship was as relatively unequivocal as it was shown to be emphasizes the strength of the link between insulin action and insulin secretion. An issue not addressed by this study, but one that cannot be ignored given the results, is the mechanism that apparently permitted these individuals to secrete additional insulin in order to maintain glucose tolerance as they became more insulin resistant. More specifically, how does the beta cell know it should secrete more insulin? It is obvious that this question cannot be answered with currently available data, and the matter is only raised to indicate an important area for future research. It seems reasonable to predict that the results of such studies would provide useful insights into the pathogenesis of non-insulin-dependent diabetes.

Although these data clearly support the view that elevated plasma insulin concentrations after a glucose challenge represent a compensatory effect to maintain euglycemia, it is apparent from the quantitative nature of the correlation noted that the relationship between these two variables is modest in magnitude. Specifically, only 37% of the variance in plasma insulin responses to oral glucose of normal subjects can be attributed to differences in insulin action. As a corollary, it is obvious that knowledge of the plasma insulin response to oral glucose of an individual provides only an approximation of how insulin acts in that subject. At the least, considerable caution should be exercised in the use of insulin responses as a means of comparing insulin action in normal subjects.

Finally, it seems necessary to comment on the approximate fivefold variation in estimates of in vivo insulin action seen in Figure 1. Thus, subjects are able to maintain normal oral glucose tolerance despite relatively enormous variations in insulin resistance. Of even greater interest is the inability to account for the differences in insulin action seen in the normal subjects we studied. Indeed, we could only account for 62% of the variance in insulin resistance when we took into account every variable that we could conceive of that might affect insulin action in vivo. Whether the residual variance is due solely to genetic differences, or to as yet unidentified environmental variables, is unknown. In either case, it is clear that our understanding of factors regulating in vivo insulin action in humans leaves a great deal to be desired.

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