Quantification of Insulin Secretion and In Vivo Insulin Action in Nonobese and Moderately Obese Individuals with Normal Glucose Tolerance

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SUMMARY

Insulin secretion and in vivo insulin action were quantified in nonobese and moderately obese subjects (approximately 35% above desirable body weight) with normal glucose tolerance. Insulin secretion was estimated by determining plasma insulin responses to a 75-g oral challenge, and in vivo insulin-stimulated glucose uptake by the euglycemic clamp technique. Plasma glucose levels of the two groups were identical during the glucose tolerance test, but the plasma insulin response was significantly greater ($P < 0.01$) in the obese subjects. However, insulin-stimulated glucose utilization by the two groups was equal during the euglycemic clamp studies. These results were supported by the fact that degree of obesity correlated significantly with insulin response ($r = 0.61$, $P < 0.005$), but not with insulin-stimulated glucose utilization ($r = -0.25$, $P > 0.2$). Thus, indirect evidence that moderately obese subjects were more insulin-resistant based on measurement of plasma insulin response was not supported by direct quantification of insulin action. One explanation for these findings is that the height of the plasma insulin response bears no relationship to loss of in vivo insulin action, but that seems unlikely in view of the fact that there was a significant correlation ($r = -0.52$, $P < 0.01$) between these two variables in the group as a whole. Therefore, it appears that the hyperinsulinemia seen in obese individuals may not be a simple function of insulin resistance, and that the ability of insulin to stimulate glucose utilization is not significantly impaired in moderately obese subjects with normal glucose tolerance. Alternatively, the degree of impairment in insulin action seen in these individuals is insufficient to be detected by the euglycemic clamp technique. DIABETES 32:600–604, July 1983.

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Approximately 20 yr ago, Karam et al.[1] pointed out that obese individuals respond to an oral glucose challenge with an exaggerated insulin response, and this observation has been confirmed on repeated occasions.[2] It has been generally assumed that the hyperinsulinemia of obese individuals is a secondary phenomenon, and represents a compensatory increase in insulin secretion aimed at overcoming the insulin resistance associated with obesity. Although there is considerable ancillary support for this view,[2] there are surprisingly little quantitative data as to the effect of obesity on in vivo insulin-stimulated glucose utilization. Furthermore, data that are available have evolved from studies of massively obese individuals.[3–7] Therefore, we initiated the experiments to be presented subsequently, in which we determined both the plasma insulin response to oral glucose and the ability of insulin to stimulate glucose utilization in nonobese and moderately obese subjects with normal glucose tolerance. The results of these studies again indicated that obesity is associated with an exaggerated insulin response to an oral glucose challenge. However, we could not document any difference in insulin-stimulated glucose utilization between the two groups. Thus, moderately obese individuals with normal oral glucose tolerance could not be shown to be more insulin-resistant than nonobese subjects, and the hyperinsulinemia seen in the moderately obese subjects may not be a simple function of a loss of normal insulin sensitivity.

MATERIALS AND METHODS

The experimental subjects, all of whom were volunteers, were admitted to the Stanford General Clinical Research Center. They consumed a weight-maintaining liquid formula diet, with a caloric distribution of 43% carbohydrate, 42% fat, and 15% protein. This diet was consumed for at least 3 days prior to any study. The daily intake was divided into three meals, containing ⅕, ⅖, ⅖ of total calories, and consumed at 0800 h, 1200 h, and 1800 h. Degree of obesity was determined by comparing actual weight with standard

TABLE 1
Clinical characteristics (mean ± SEM)

	Height (cm)	Weight (kg)	SA* (m^2)	RBW† (%)	FPG‡ (mg/dl)	FPI§ (μU/ml)	Age (yr)	Sex (M/F)
Nonobese	170 ± 3	66.1 ± 3.4	1.76 ± 0.06	100 ± 2	87 ± 2	8 ± 1	41.6 ± 4.5	4/6
Obese	171 ± 2	91.1 ± 5.0	2.03 ± 0.06	136 ± 6	91 ± 2	12 ± 2	40.7 ± 4.5	4/6
	NS	$P < 0.001$	$P < 0.001$	$P < 0.001$	NS	NS	NS	NS

*SA = surface area.
†RBW = relative body weight.
‡FPG = fasting plasma glucose.
§FPI = fasting plasma insulin.

tables of desirable body weight.[8] Subjects were defined as having normal glucose tolerance according to recently suggested critieria.[9] Participants were not taking any medication, nor had any medical problems known to affect glucose metabolism. Hepatic, renal, and thyroid functions were normal. Nonobese subjects were less than 15% above desirable body weight (range = 91–114%), and moderately obese subjects were at least 20% above desirable body weight (range = 120–163%). There were ten subjects in each group, and the two groups were carefully matched for age (range = 24–64 yr), sex, and height. These data appear in Table 1, and document the similarity of the two groups in terms of physical characteristics other than weight.

Insulin secretion was estimated by measuring plasma insulin concentration following a 75-g oral glucose challenge.[9] Glucose was given at 0800 h following an overnight fast, and blood removed for determination of plasma glucose and insulin concentrations before, and 30, 60, 120, and 180 min after the glucose administration.

In vivo insulin action was estimated by the insulin clamp technique. Since this method has been previously described in detail,[10] only the general procedure will be outlined. Blood samples were obtained from an indwelling catheter in a hand vein, kept in a radiant warmer at 70°C to provide "arterialized" samples. 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 the baseline plasma glucose concentration, a primed continuous infusion of insulin (40 mU/m^2/min) was started. Plasma glucose was determined every 5 min. A variable infusion of glucose 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 20 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 size.[10]

The use of the glucose clamp to assess glucose utilization is based on the assumption that the amount of glucose infused to maintain basal glucose levels is equal to the rate of glucose utilization. However, this is the case only when hepatic glucose production is suppressed. In order to quantify glucose utilization rate, total glucose turnover must also be determined during the clamp studies. This was done by modification of techniques previously described from our laboratory,[11] 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 for a total of 5 h. Aliquots of plasma were precipitated with $BaOH_2$ and $ZnSO_4$ at 20-min intervals, centrifuged, and the protein-free supernatant evaporated in a scintillation vial. Plasma glucose concentration 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 the insulin infusion, using the non-steady-state equation of Steele.[12] Ra and Rd should be equal during the period before the administration of insulin, and be equal to hepatic glucose output. Subtraction of the glucose infusion rate from the value of Ra during the clamp study yields hepatic glucose output (HGO) under the condition of hyperinsulinemia, and comparison of these two values defines the degrees to which insulin inhibited HGO. Essential total inhibition of HGO was seen in all studies, obviating the need to correct M for residual HGO.

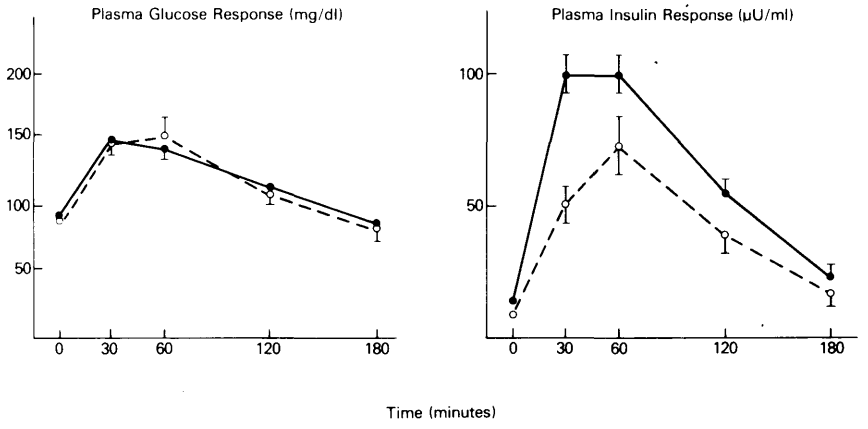

FIGURE 1. Mean (±SEM) plasma glucose and insulin responses to an oral glucose challenge in nonobese (○—○) and moderately obese (●—●) subjects with normal glucose tolerance.

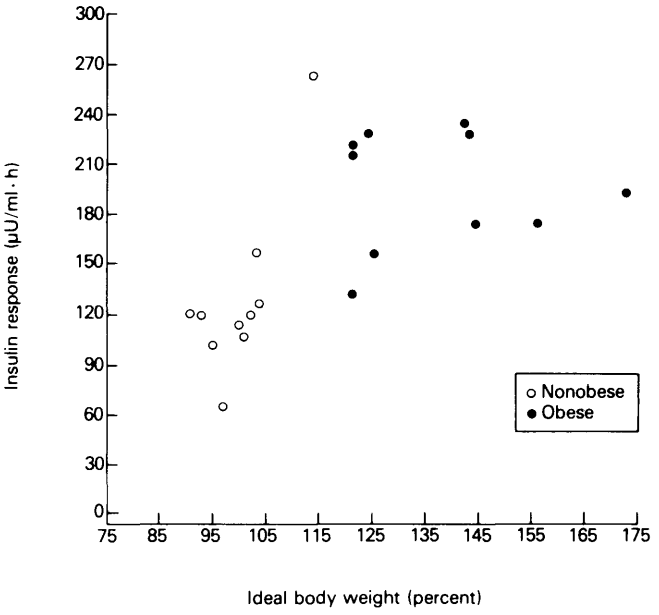

FIGURE 2. Relationship between desirable body weight and insulin response in nonobese (○) and moderately obese (●) subjects (r = 0.61, P < 0.005).

The sequence of the two tests was randomized, and they were performed 3 days apart. Plasma glucose[13] and insulin concentration[14] were measured by standard methods. Statistical analysis was performed using the Statistical Package for the Social Sciences.

RESULTS

The plasma glucose and insulin responses of the two groups to the oral glucose challenge are seen in Figure 1. The results in the left panel indicate that the plasma glucose responses of the two groups were essentially identical. In contrast, the data in the right panel demonstrate that the plasma insulin levels of the moderately obese group were higher at every time point. The differences at the individual time points were statistically significant in the fasting state ($P < 0.01$), and 30 min ($P < 0.01$) after oral glucose administration. In addition, the total insulin response (area under the curve) of the moderately obese individuals (194 ± 11 μU/ml · h) was also significantly higher ($P < 0.01$) than in the nonobese individuals (128 ± 16 μU/ml · h).

In an effort to define the relationship between insulin response to glucose and relative degree of obesity within the entire 20 subjects, we calculated the degree of correlation between the two variables. These data are seen in Figure 2, and indicate that there was a highly significant relationship ($r = 0.61$, $P < 0.005$) between degree of obesity and magnitude of the total plasma insulin response. Furthermore, this correlation only declined to 0.58 when differences in insulin-stimulated glucose utilization as measured by the insulin clamp technique were taken into account (partial correlation coefficient). Thus, there was a direct correlation between magnitude of obesity and insulin response, which appeared to be independent of differences in insulin action.

The effect of a moderate degree of obesity on insulin-stimulated glucose utilization is seen in Figure 3. The data in the left panel compare the amount of glucose utilized (M) by the two groups during a 120-min period of sustained hyperinsulinemia. Although the mean value of the moderately obese individuals (194 ± 22) was somewhat lower than that of nonobese subjects (166 ± 16), the difference was modest in magnitude, and not statistically significant. Since the data in the right panel of Figure 3 indicate that steady-state plasma insulin levels were comparable during these studies, these results do not document a significant loss of insulin action in moderately obese individuals.

Figure 4 illustrates the relationship between degree of obesity and insulin-stimulated glucose utilization for all 20 subjects, and is consistent with the view that moderate obesity was not associated with a significant loss ($r = -0.25$, $P > 0.2$) in insulin-stimulated glucose utilization.

Since it is generally believed that the hyperinsulinemia seen in obese individuals is secondary to their insulin resistance, it was surprising to find a correlation between obesity and insulin response (Figure 2), but not between obesity and insulin-stimulated glucose uptake (Figure 4). However, a paradox only exists if the plasma insulin response is a reflection of the ability of insulin to stimulate glucose disposal, and this issue is addressed in Figure 5. These data indicate that the lower the amount of glucose utilized during the period of sustained hyperinsulinemia (the more "insulin-resistant"), the greater the insulin response ($r = -0.53$, $P < 0.02$). Thus, there was a significant correlation between the indirect (insulin response) and direct (euglycemic clamp) measurement of insulin resistance in the entire population.

DISCUSSION

These data confirm earlier observations[2] that obese individuals with normal glucose tolerance have an exaggerated insulin response to glucose, and demonstrate that this is also true of moderately obese subjects. However, our results fail to confirm the assumption that this phenomenon can be attributed to the fact that these individuals are more resistant to the ability of insulin to stimulate glucose utilization. Thus, there was no significant difference between estimates of insulin-stimulated glucose utilization in nonobese and moderately obese subjects (Figure 3). Furthermore, we could not document a significant relationship between degree of obesity and insulin action (Figure 4). An obvious explanation for

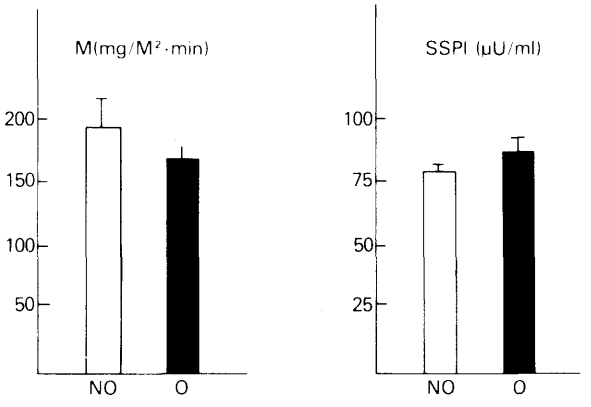

FIGURE 3. Mean (±SEM) insulin-stimulated glucose utilization rates (M) and steady-state plasma insulin levels during the euglycemic clamp in nonobese (NO) and moderately obese (O) subjects.

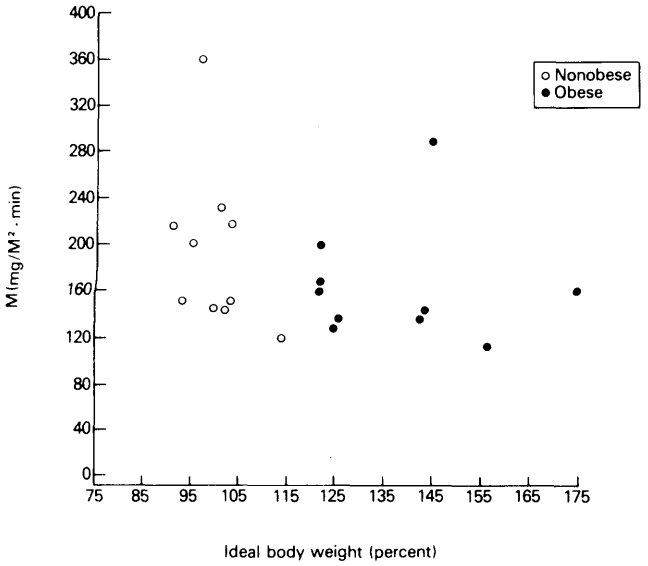

FIGURE 4. Relationship between desirable body weight and insulin-stimulated glucose utilization (M) in nonobese (○) and moderately obese (●) subjects (r = −0.25, P > 0.2).

this apparent discrepancy is that there need not be a relationship between insulin response to glucose and insulin-stimulated glucose utilization. However, the data in Figure 5 indicate that such a relationship did exist ($P < 0.02$) between these two variables in our study population. On the other hand, the magnitude of the relationship was relatively modest in magnitude ($r = -0.53$), and many variables, other than degree of insulin resistance, can modulate the height of the plasma insulin response to a glucose challenge. For example, variations in the secretion of GI factors in response to oral glucose could certainly account for differences in plasma insulin level, independently of changes in insulin action. Additionally, differences in insulin catabolic rate can also play a regulatory role in determining the height of the plasma insulin response to glucose. Indeed, it has been noted that perfused livers from obese mice[15] and rats[16] do not remove insulin as efficiently as livers from nonobese animals. However, we could not document any difference between the two groups in the steady-state insulin levels achieved during the infusions. Furthermore, the correlation between desirable body weight and steady-state plasma insulin level during the infusion was not significant ($r = 0.22$, $P > 0.2$), nor were there differences in the metabolic clearance rates of insulin between the two groups. It is obvious that this possibility deserves further consideration, but it doesn't appear to account for higher insulin levels in the moderately obese group following oral glucose. Although one might comment on other attributes of obesity which could play a role in the development and/or maintenance of higher insulin levels, e.g., failure of insulin to suppress beta cell function,[17] we did not measure C-peptide levels, and this does not appear to be a useful speculation in the absence of the pertinent data. Suffice it to say at this point that there are many possible explanations for why moderately obese individuals could have higher insulin levels in response to an oral glucose challenge and not be more insulin-resistant than nonobese subjects.

An alternative possibility for our failure to document a loss of insulin-stimulated glucose utilization in the moderately obese subjects is that our methods were inadequate. For example, the insulin clamp technique is not simple to perform. On the other hand, we have had considerable experience with its use,[18,19] and the clamps were technically excellent by all the usual criteria.[10] However, the fact that the insulin clamps were technically excellent does not necessarily mean that this method of estimating in vivo insulin action is sensitive enough to discern differences that might exist between nonobese and moderately obese subjects with normal glucose tolerance. For example, we have previously indicated that the investigator is capable of inducing changes in estimates of insulin action of approximately 20% within the same individual.[18] In addition, estimates of insulin action generated by the insulin clamp depend on the standard of reference. We have chosen to express glucose utilization on the basis of surface area, and it can be easily appreciated that estimates of insulin action based simply upon weight would have resulted in a greater difference between the values for M in the two groups of subjects. On the other hand, it could be argued that M should be expressed in terms of lean body mass, and this might lead to the conclusion that insulin-stimulated glucose utilization was actually greater in the moderately obese subjects. All of these considerations serve to emphasize the tentative nature of our conclusions concerning the effect of moderate obesity on insulin action, and it is certainly possible that marginal decreases in insulin-stimulated glucose utilization could have existed in the moderately obese subjects which were undetectable by the technique used. Indeed, the only argument against this conclusion is the fact that the five previous studies addressing this issue[3-7] all found a significant reduction in insulin action in obese patients. However, the subjects were considerably more obese in the earlier studies, i.e., 193%,[3] 176%,[5] 199%,[6] and 156%[7] above desirable body weight, and were not matched for degree of glucose

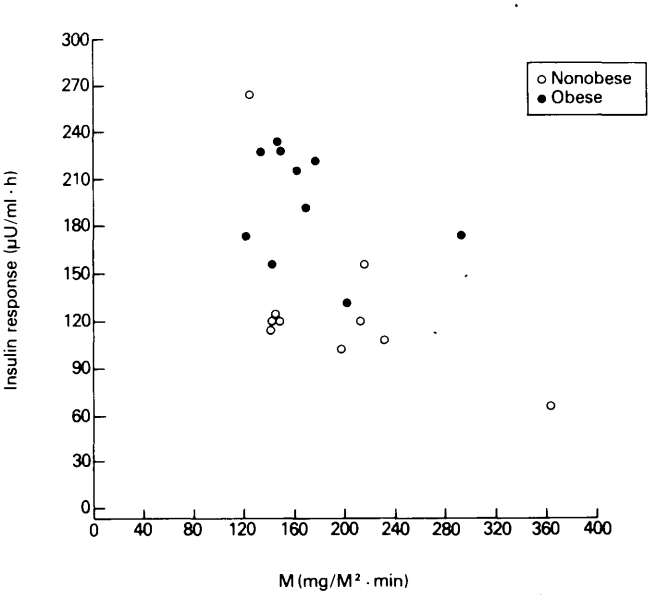

FIGURE 5. Relationship between insulin-stimulated glucose utilization rates (M) and insulin responses to the glucose challenge in nonobese (○) and moderately obese (●) subjects (r = −0.53, P > 0.02).

tolerance. For example, Burnand and associates[6] did not perform oral glucose tolerance tests, and it appears that only 6 of 13 patients studied by Kolterman et al.[5] had normal glucose tolerance. Similarly, only two of the five patients studied by Vranic et al.[6] were said to have normal glucose tolerance. Since glucose intolerance alone increases severity of insulin resistance,[11,18,20] it is obvious that the effect of obesity on in vivo insulin action can only be evaluated when subjects are matched for glucose tolerance. When analyzed in this fashion, it is clear that our results are not as discordant with previous data as might have initially appeared, and it is not unreasonable that any differences in insulin-stimulated glucose utilization between nonobese and moderately obese subjects that might exist would be modest in magnitude. As such, our inability to document this difference with a relatively crude technique might not be unexpected.

Finally, the fact that we could not document any difference in in vivo insulin action between nonobese and moderately obese subjects when matched for age and degree of glucose intolerance should not be interpreted as indicating that changes in degree of obesity will not affect these variables. In the first place, our patients were not massively obese. Secondly, we did not perform full insulin dose-response curves, and it is possible that glucose utilization would be lower if obese subjects were compared with nonobese subjects at much higher steady state insulin levels. (Parenthetically, one could also question the significance of differences in the ability of insulin to stimulate in vivo glucose uptake at insulin levels far beyond those that occur in vivo). Furthermore, there is certainly evidence that in vivo insulin action improves with weight loss.[21] Thus, we believe that our results are entirely consistent with the view that obesity plays a modulating role,[21] i.e., insulin sensitivity may deteriorate with the development of obesity, but an insulin sensitive subject won't necessarily be transformed into an insulin-resistant subject by moderate weight gain.

In conclusion, our results indicate that moderately obese subjects with normal glucose tolerance are hyperinsulinemic as compared with nonobese individuals with similar glucose tolerance. In contrast, the two groups of subjects had comparable rates of insulin-stimulated glucose utilization. These data indicate that moderately obese subjects are, at most, only slightly more insulin-resistant than are nonobese subjects when the groups are carefully matched for age and glucose tolerance.

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