Roles of insulin resistance and obesity in regulation of plasma insulin concentrations

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Roles of insulin resistance and obesity in regulation of plasma insulin concentrations. Am. J. Physiol. Endocrinol. Metab. 278: E501–E508, 2000.—Plasma glucose, insulin, and C-peptide concentrations were determined in response to graded infusions of glucose, and insulin secretion rates were calculated over each sampling period. Measurements were also made of insulin clearance, resistance to insulin-mediated glucose uptake, and the plasma glucose, insulin, and C-peptide concentrations at hourly intervals from 8:00 AM to 4:00 PM in response to breakfast and lunch. Plasma glucose, insulin, and C-peptide concentrations were significantly (P < 0.01) higher in obese women in response to the graded intravenous glucose infusion, associated with a 40% (P < 0.005) greater insulin secretory response. Degree of insulin resistance correlated positively (P < 0.05) with the increase in insulin secretion rate in both nonobese (r = 0.52) and obese (r = 0.58) groups and inversely (P < 0.05) with the decrease in insulin clearance in obese (r = −0.46) and nonobese (r = −0.39) individuals. Weight loss was associated with significantly lower plasma glucose, insulin, and C-peptide concentrations in response to graded glucose infusions and in day-long insulin concentrations. Neither insulin resistance nor the insulin secretory response changed after weight loss, whereas there was a significant increase in the rate of insulin clearance during the glucose infusion. It is concluded that 1) obesity is associated with a shift to the left in the glucose-stimulated insulin secretory dose-response curve as well as a decrease in insulin clearance and 2) changes in insulin secretion and insulin clearance in obese women are more a function of insulin resistance than obesity.

Insulin secretion; weight loss

We have recently published evidence that the hyperinsulinemia associated with insulin resistance in nonobese healthy women results from an increase in insulin secretion, secondary to a shift to the left of the glucose-stimulated insulin response curve, as well as a decrease in insulin clearance (11). Insulin resistance and hyperinsulinemia are associated with obesity, and both abnormalities improve after weight loss (16). Although the effects of obesity and weight loss on various facets of insulin metabolism have been the focus of several recent reports (10, 13, 17, 18, 20), none of them have examined the dose-response relationships between glucose and insulin secretion (2, 3). Furthermore, the published studies have not attempted to define the relative roles played by obesity, as contrasted to insulin resistance, in the hyperinsulinemia associated with obesity. Consequently, we initiated the current study in which we have used a graded intravenous glucose infusion (2, 3) in nondiabetic women to define the effect of obesity and weight loss on the dose-response relationship between plasma glucose concentration and insulin secretion.

**METHODS**

The experimental population consisted of 18 obese [body mass index (BMI) > 29] nondiabetic healthy women < 60 yr of age, recruited from the San Francisco Bay area by advertisements in local newspapers. They were compared with 20 nonobese (BMI < 26) women of similar age distribution who had been studied previously (11). All subjects were judged to be in good general health on the basis of history, physical examination, complete blood count, routine biochemical screening, and electrocardiogram and had normal oral glucose tolerance on the basis of National Diabetes Data Group criteria (15). They were normotensive (blood pressure < 160/90) and were taking no medication known to affect insulin secretion or sensitivity. This project was approved by the Stanford Human Subjects Committee, and all women gave written informed consent.

At baseline, each subject was admitted to the clinical research center for the tests performed in the following order:

**Test 1.** Pancreatic β-cell function was quantified by determining the insulin and C-peptide response to graded intravenous infusions of glucose (2, 3, 11). After an overnight fast, intravenous catheters were placed in superficial antecubital veins in each arm. One arm was used for infusion of 20% glucose. This was started at a rate of 1 mg·kg⁻¹·min⁻¹, followed by infusions of 2, 3, 4, 6, and 8 mg·kg⁻¹·min⁻¹. Each infusion was administered for a period of 40 min. Venous blood samples for glucose (12), insulin (6), and C-peptide (9) were obtained from the contralateral arm at fasting and then 10, 20, 30, and 40 min into each glucose infusion period. The last two values at the end of each infusion period were averaged and used as the mean for that infusion.

Insulin secretion rates over each sampling period were derived by deconvolution of peripheral plasma C-peptide concentrations, using a two-compartment model of C-peptide kinetics and standard parameters for C-peptide clearance estimated for each subject, taking into account body surface area and body weight. The results are expressed as the mean ± SD. Statistical analyses were performed using the paired t-test and the two-tailed Student's t-test. The significance level was set at P < 0.05. Statistical analyses were performed using the paired t-test and the two-tailed Student's t-test. The significance level was set at P < 0.05.
area and age. For each subject, the mean glucose and insulin concentrations during each of the six glucose infusion periods were plotted against each other, allowing an insulin-glucose dose-response curve to be constructed. The best-fit quadratic curve (least squares fit using Microsoft Deltagraph) drawn through the data was used to compare each subject’s plasma insulin response at the same glucose level. The insulin concentration at molar increments of plasma glucose beginning at 5 mM was then obtained by interpolation. The same technique applied to the insulin secretion rate (although in this case a straight line provided the best fit) defines the insulin secretion rate at molar increments of plasma glucose for each subject. This method for assessing insulin secretion has been validated and has been in use for the past several years to demonstrate alterations in the relationship between changes in glucose concentration and insulin secretion (2, 3, 11, 22).

It should be noted that venous, not “arterialized,” blood was used in the calculation of insulin secretion, and the appropriateness of this decision could be questioned in light of the suggestion by Marks (14) that “the glucose concentration in venous blood may be 2–3 mmol·l⁻¹ lower than in arterial blood” in a “glucose-loaded subject.” However, the reference cited by Marks was published in 1923 (4), used a nonspecific method (Folin-Wu) for measuring plasma glucose concentration, and was based on a comparison of venous versus finger-stick blood. We have compared data obtained using mixed venous blood versus arterialized blood in hyperinsulinemic clamp studies (1). Measurements of plasma insulin and glucose concentrations were essentially identical, irrespective of whether or not arterialized or mixed venous blood was used. This was true of glucose concentrations during the clamp, and no great change in the glucose disposal rate (M) value was noted. Perhaps the crucial distinction is between the unsteady state in response to an acute glucose load compared with the steady-state conditions achieved in response to a continuous infusion. In the latter situation, any arterio-venous concentration difference should be constant throughout the study. Finally, the crucial values are the concentrations of insulin and glucose in the interstitial fluid. Indeed, it is possible that venous blood may provide a more reasonable index of the interstitial concentrations of insulin and glucose. Thus we do not believe that the results are significantly confounded by our use of venous blood.

Test 2. Resistance to insulin-mediated glucose disposal was estimated by a modification (7) of the original insulin suppression test (5, 21). After an overnight fast, intravenous catheters were placed in a superficial antecubital vein in each arm. One arm was used for a continuous 180-min infusion of glucose (240 mg·m⁻²·min⁻¹), sandostatin (octreotide acetate: bolus of 25 μg followed by 0.5 μg·m⁻²·min⁻¹), and insulin (25 mU·m⁻²·min⁻¹). Venous blood samples for glucose and insulin determinations were obtained from the contralateral arm every 30 min (to 150 min) and then every 10 min for the last 30 min of the infusion. The mean of these last four values was used to calculate the steady-state plasma glucose (SSPG) and insulin (SSIPI) concentrations. Under these experimental conditions, endogenous insulin secretion is suppressed by sandostatin, and the SSIPI concentration achieved is comparable in all individuals. The SSPG provides a measure of insulin-mediated glucose disposal; the higher the SSPG, the more insulin resistant the individual. Insulin resistance as assessed by the insulin suppression test has been shown to correlate almost perfectly (r > 0.9) with values of insulin resistance achieved with the insulin clamp technique (5).

Test 3. In addition, the obese volunteers received an 8-h meal tolerance test consisting of two meals, breakfast served immediately after fasting blood samples were drawn at ~8:00 AM and lunch served 4 h later. Breakfast contained 20% and lunch 40% of the individual’s caloric requirements, and the macronutrient content of each meal was 43% carbohydrate, 15% protein, and 42% fat. Blood was drawn at hourly intervals for 8 h, and plasma was frozen for measurement of glucose and insulin concentrations.

Weight loss began the day after the first meal tolerance test. The Harris-Benedict equation (8) was used to determine each volunteer’s total caloric requirements. One thousand calories was subtracted from their total caloric requirements to determine daily caloric intake during the weight loss phase of the study. No one received <1,200 kcal/day. A commercial canned liquid nutritional formula, plus two high-fiber muffins per day, provided their diet for 9 wk. Each volunteer came into the research unit two times a week for measurement of body weight and to pick up their liquid formula and muffins. The hypocaloric diet was effective, with an average weight loss of 9.8% of baseline weight.

At the end of the weight loss phase, each volunteer followed a weight maintenance diet for 1 wk before admission to the research center for a repeat of the baseline tests (insulin suppression test, graded glucose infusion, and meal tolerance test). To minimize assay variability, samples from before and after weight loss were determined in the same assay. Values for continuous variables are expressed as means ± SE. The SSPG and SSIPI values were compared using two-tailed, paired Student’s t-tests. Differences between the glucose and insulin profiles after the meal tolerance test and the insulin secretion response to intravenous glucose in the two groups were compared by two-way ANOVA with SAS software. The integrated plasma insulin and insulin secretory responses and plasma insulin clearances were compared using Student’s t-test, paired or unpaired as appropriate.

RESULTS

The obese and nonobese women were matched for age (42 ± 2 vs. 41 ± 2 yr) but differed by BMI (31.9 ± 0.4 vs. 22.5 ± 0.4 kg/m²). The obese women had higher SSPG concentrations than their nonobese counterparts (9.22 ± 0.99 vs. 5.87 ± 0.71 mmol/l, P < 0.01), but there was considerable overlap between the two groups. Mean plasma glucose, insulin, and C-peptide concentrations achieved at each stage of the graded glucose infusion are illustrated in Fig. 1. These results show that the obese women had a slightly higher plasma glucose concentration (averaging ~1.1 mmol/l higher) at any given glucose infusion rate (P < 0.01 by 2-way ANOVA). Despite this relatively small difference in plasma glucose concentrations, it is apparent that the plasma insulin concentrations at the end of each glucose infusion were much higher in the obese subjects (P < 0.001 by ANOVA). The relative increase in the plasma C-peptide concentrations was of comparable magnitude in the obese women (P < 0.01 by ANOVA).

The plasma insulin concentrations and insulin secretion rates at molar increments of plasma glucose are shown in Fig. 2. The obese subjects had higher plasma insulin concentrations and insulin secretion rates as the plasma glucose concentration was increased above 5 mmol/l, and in both cases the increases were statistically significant (P < 0.01 by ANOVA). The magnitude of these differences can be evaluated by comparing the total integrated responses as the plasma glucose concen-
In the case of plasma insulin, there was a 60% increase in the response of the obese individuals (insulin area under the curve: obese vs. nonobese, 660 ± 62 vs. 415 ± 43 pM/mM, P < 0.01 by t-test), whereas the increase in insulin secretion rate was 40% in these subjects (insulin secretion area under the curve: obese vs. nonobese was 1,612 ± 107 vs. 1,148 ± 104 pmol·min⁻¹·mM, P < 0.005 by t-test). Finally, the slope of the relationship between incremental increases in plasma glucose and insulin secretion was significantly greater (P < 0.05) in obese compared with nonobese subjects (121 ± 15 vs. 85 ± 10 pmol·min⁻¹·mM⁻¹). The observation that the insulin response tended to increase to a greater extent than did the insulin secretion rate raised the possibility that insulin clearance rate might be lower in obese individuals. Although endogenous insulin clearance [endogenous metabolic clearance rate (MCR) adjusted for body surface area] calculated as the ratio of the total secretion of insulin to the area under the peripheral insulin curve was lower in the obese subjects (1.41 ± 0.1 vs. 1.61 ± 0.13 l·min⁻¹·m⁻²), this difference was not statistically significant (P = 0.25).

Fig. 1. Plasma glucose (A), insulin (B), and C-peptide (C) concentrations at each stage of the graded glucose infusion in the obese (●) and nonobese (□) groups.

Fig. 2. Plasma insulin concentrations (A) and insulin secretion rates (B) in response to molar increments of plasma glucose concentration during the graded glucose infusion in the obese (●) and nonobese (□) groups.
Figure 3 shows the relationship between insulin resistance (as measured by SSPG), plasma insulin concentration, insulin secretion rate, and plasma insulin clearance as the plasma glucose concentration was increased from 5 to 9 mmol/l. Integrated plasma insulin concentrations and insulin secretion rates (area under the dose-response curves) were highly correlated ($P < 0.001$) with SSPG concentration (correlation coefficients 0.77 and 0.66, respectively, for the total group of women). Furthermore, the relationship between SSPG and the integrated insulin response remained statistically significant ($P < 0.005$) when nonobese ($r = 0.78$) and obese ($r = 0.67$) subjects were considered separately. Similarly, the relationship between SSPG and insulin secretion rate was also statistically significant ($P < 0.05$) within the two subgroups, with $r$ values of 0.52 and 0.58 in nonobese and obese women, respectively. In marked contrast, the relationship between BMI and insulin response was not significantly correlated in either nonobese ($r = 0.20$) or obese ($r = -0.22$) women, nor were BMI and insulin secretion rates related (0.18 and $-0.24$ in nonobese and obese women).

Insulin clearance, calculated as defined above, was negatively correlated with SSPG ($r = -0.46$, $P < 0.05$), as also shown in Fig. 3. The relationship between SSPG and insulin clearance was present ($P < 0.05$) in both the nonobese ($r = -0.49$) and obese ($r = -0.39$) groups. However, as before, there was no relationship between BMI and insulin clearance in either weight group ($r = -0.13$ and 0.19).

Fourteen women successfully completed the weight loss phase of the study, losing an average of 9.8% of initial body weight in 9 wk. Figure 4 displays the changes in SSPG concentration before and after weight loss. It can be seen that SSPG concentrations varied approximately sixfold in these obese women before weight loss and that a fall was observed in 10 of the 13 women in whom both values were available. However, in three instances, SSPG concentrations were actually higher after weight loss. As a consequence, the mean decrease in SSPG concentration associated with weight loss was not significant ($8.6 \pm 1.1$ vs. $9.9 \pm 1.1$, mmol/l, $P = 0.10$). On the other hand, SSP1 concentrations were also slightly lower ($P < 0.09$) after weight loss ($344 \pm 19$ vs. $380 \pm 25$ pmol/l), and this may have minimized the improvement in SSPG associated with weight loss.

The results of the meal tolerance tests before and after weight loss are also shown in Fig. 4. There was a small nonsignificant decrease in plasma glucose during the 8 h of the test, averaging 0.2 mmol/l. However, the day-long plasma insulin concentrations were significantly lower after weight loss ($P < 0.05$ by ANOVA).

Mean plasma glucose, insulin, and C-peptide concentrations achieved at each stage of the graded glucose infusion before and after weight loss are shown in Fig. 5. These results show that weight loss led to a modest but significant decrease in plasma glucose of $\sim 0.42$ mmol/l at any given glucose infusion rate ($P < 0.05$ by ANOVA). The plasma insulin concentrations at the end of each glucose infusion rate were 38% lower and the plasma C-peptide concentrations 18% lower after weight loss ($P < 0.01$ by ANOVA).

The effect of weight loss on the plasma insulin concentrations and insulin secretion rates at molar
increments of plasma glucose are shown in Fig. 6. Although the plasma insulin concentrations were clearly decreased after weight loss, the insulin secretion rate was only minimally and nonsignificantly lower. The magnitude of these differences can be evaluated by comparing the total integrated responses as the plasma glucose concentration was increased from 5 to 9 mmol/l. In the case of plasma insulin, there was a 30% decrease after weight loss (insulin area under the curve: before vs. after 700 ± 46 pM/mM, P = 0.005 by paired t-test), whereas there was little change in insulin secretion rate (insulin secretion area under the curve: before vs. after 1,560 ± 129 vs. 1,464 ± 132 pmol·min⁻¹·mM; not significant).

The observation that there was a decrease in plasma insulin concentration after weight loss without a corresponding decrease in insulin secretion rate strongly suggested that there was an increase in the rate of insulin clearance, a possibility supported by the fact that there was a decrease in SSPI concentrations after weight loss. Calculating insulin clearance as the ratio of the total production of insulin to the area under the peripheral insulin curve confirmed this (endogenous MCR adjusted for body surface area: before vs. after 1.23 ± 0.07 vs. 1.75 ± 0.09 l·min⁻¹·m⁻², P < 0.001 by paired t-test). To further evaluate the effect of weight loss on insulin clearance, we calculated the exogenous MCR by using the SSPI concentrations obtained during the insulin suppression test. In this case, the increase with weight loss was of lesser magnitude (0.48 ± 0.18 vs. 0.37 ± 0.15 l·min⁻¹·m⁻², P < 0.05).

**DISCUSSION**

These studies were designed to explore the dose-response relationships between plasma glucose concentration and insulin secretion rates in response to intravenous glucose and were initiated for two reasons. In the first place, we wished to quantify the effect of obesity on the insulin secretory response to a graded intravenous glucose infusion in healthy normal glucose-tolerant females. The results presented have shown that the plasma insulin and C-peptide responses were significantly higher in response to the glucose infusion in obese compared with nonobese women studied previously (11), and the increment in the two variables was comparable. In addition, obesity was associated with an ~40% increase in the insulin secretory response. The evidence that the glucose-stimulated insulin dose-response curve was shifted to the left in obese women is consistent with the conclusions from earlier studies (10, 13, 17, 18, 20), using a variety of different experimental approaches, that the hyperinsulinemia associated with obesity is primarily due to increased insulin secretion. However, it should be emphasized that the increases in plasma insulin and C-peptide concentrations, as well as insulin secretion rates, seen in the obese women were markedly accentuated compared with the much more modest increase in plasma glucose...
concentration during the infusion study. In other words, the stimulus to the pancreatic β-cells to secrete more insulin in these obese women cannot be a simple function of the coexisting plasma glucose concentration. Rather, it appears that there is an increase in the sensitivity of the pancreatic β-cells to a given increment in plasma glucose concentration in obese women, a phenomenon quite similar to that we recently described in nonobese, insulin-resistant women (11).

The other goal of this study was to define the relationship between changes in plasma glucose concentration and insulin secretion associated with weight loss. In this instance, the answer may not be as straightforward as it might seem. The simplest answer would be that weight loss had little, if any, effect on insulin secretion. This conclusion can be inferred by the observation that the decrease in the plasma insulin response to the graded glucose infusion was approximately two times as great as the fall in C-peptide. More specifically, there was essentially no change in the insulin secretory response to the graded glucose infusion after weight loss, whereas insulin clearance was significantly increased. On the other hand, in our previous study (11) we emphasized the importance of insulin resistance in determining the insulin secretory response to a graded concentration during the infusion study. In other words, the stimulus to the pancreatic β-cells to secrete more insulin in these obese women cannot be a simple function of the coexisting plasma glucose concentra-

Fig. 5. Plasma glucose (A), insulin (B), and C-peptide (C) concentrations at each stage of the graded glucose infusion before (●) and after (○) weight loss.

Fig. 6. Plasma insulin concentrations (A) and insulin secretion rates (B) in response to molar increments of plasma glucose concentration during the graded glucose infusion before (●) and after (○) weight loss.
glucose infusion. As the results in Fig. 4 indicate, a large segment of our obese group was not very insulin resistant. Furthermore, the results in Fig. 4 show that the improvement in insulin resistance associated with a decrease in baseline body weight of \(-10\%\) was modest in magnitude. In light of these considerations, the most reasonable conclusion would be that weight loss, per se, primarily affects insulin clearance, whereas a decrease in insulin secretion with weight loss may be more dependent on an associated improvement in insulin resistance.

The conclusion that the change in insulin secretion after weight loss may be primarily related to associated changes in insulin resistance is consistent with the results of both the current study and previous observations (10, 13, 18, 20). The great variability in the SSPG concentration of the obese women in the current study was also seen in the nonobese women. However, irrespective of the degree of obesity, SSPG concentration was highly correlated with both the integrated insulin response to the graded glucose infusion and the glucose-stimulated insulin secretory response. These relationships were seen when the obese and the nonobese groups were considered separately and when the two subgroups were combined. In contrast, we could not detect a significant relationship between BMI, the estimate of degree of obesity, and either insulin response or secretion in either group. As such, these data strongly suggest that it is insulin resistance, rather than obesity, that is primarily responsible for the increased insulin secretion and hyperinsulinemia seen in obese individuals. Because obese individuals, as a group, tend to be insulin resistant (16, 20), it is not surprising that they, as a group, secrete more insulin and are hyperinsulinemic. These data provide further evidence that insulin resistance leads to a leftward shift in the glucose-stimulated insulin dose-response curve and support the view that a rightward shift after weight loss would depend on an improvement in insulin sensitivity.

Although our results may seem somewhat disparate from the results of earlier studies, the differences can be easily reconciled. Thus Jimenez et al. (10) found that insulin secretion did not appear to increase after gastroplasty in six obese subjects who lost an average of 22% of initial body weight. Indeed, it required further weight loss of another 14% of initial body weight before there was any reduction in insulin secretion. Similarly, a mean weight loss of 30 kg after gastroplasty in the study by Letievre and associates (13) “slightly reduced insulin secretion but markedly improved insulin clearance.” Thus, given the fact that the weight loss in our patients was much less in magnitude than in either of the above studies, it should not be too surprising that we did not see a significant increase in insulin secretory response. Perhaps, the results most similar to the current findings are those of Polonsky et al. (18), who found that both basal and 24-h insulin secretion rates decreased after weight loss in obese subjects with normal oral glucose tolerance or with a diabetic glucose tolerance test. However, in the same study, it was apparent that the insulin secretion rates in the more insulin-resistant group, those with the abnormal glucose tolerance tests, were highest at baseline and fell the most with weight loss. These results lend further support to the importance of insulin resistance in regulation of insulin secretion.

In conclusion, the peripheral hyperinsulinemia associated with obesity in normal glucose-tolerant individuals is due primarily to an increase in the glucose-stimulated insulin dose-response curve. However, this change seems to be primarily a function of the fact that insulin resistance is common in obese individuals, and the more insulin resistant an individual, the more sensitive the pancreatic \(\beta\)-cell to increments in plasma glucose concentration. An increase in insulin clearance appears to occur after weight loss of \(-10\%\) of initial body weight, whereas decreases in insulin secretion seem to require a greater degree of weight loss and/or an improvement in insulin sensitivity. Perhaps this difference in the observed effects of weight loss on insulin clearance and insulin secretion is due to the fact that weight loss in obese individuals will relatively uniformly lead to an increase in insulin clearance, but any change in pancreatic \(\beta\)-cell sensitivity to glucose is dependent on an improvement in insulin resistance. Finally, the insights gained from this study provide further support for the utility of the graded glucose infusion approach to illuminate how glucose-stimulated secretion is regulated.

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