Hyperinsulinemia in Individuals with Obesity: Role of Insulin Clearance

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Objective: Several studies have shown decreased insulin clearance rate (ICR) in individuals with obesity, but it remains unclear whether this is predominately due to obesity-associated insulin resistance (IR) or obesity itself. This study aimed to clarify the complex interrelationship that exists between obesity, IR, and ICR.

Methods: Healthy volunteers (n = 277) had measurement of IR and ICR using the insulin suppression test (IST). IR was quantified by determining the steady-state plasma glucose (SSPG) during the IST. ICR was estimated by dividing the insulin infusion rate by the steady-state plasma insulin concentration. We performed our analysis by stratifying the experimental population into four dichotomous categories, varying in obesity and IR. Obesity was defined as a body mass index (BMI) ≥ 30 kg/m², and IR was defined as SSPG ≥ 150 mg/dL.

Results: Individuals with obesity had higher fasting insulin compared with individuals without obesity, regardless of IR. ICR was similar between individuals with and without obesity but was higher in insulin resistant individuals compared with insulin-sensitive individuals. In multivariate analysis, both fasting insulin and SSPG were significantly associated with ICR. No significant relationships were observed between BMI and ICR.

Conclusions: Reduced ICR in obesity is secondary to IR, not excess adiposity.

Introduction

There is general agreement (1-3) that hyperinsulinemia in individuals with obesity is related to increases in insulin secretion and decreases in insulin clearance rate (ICR). Individuals with obesity tend to be insulin resistant, and there is substantial evidence that insulin resistance (IR) is also associated with an increase in insulin secretion and a decrease in ICR (1-3). What remains controversial is whether increased insulin secretion and decreased ICR in individuals with obesity are primarily a function of their IR, as compared to the view that obesity, per se, independent of IR, can decrease insulin clearance (1-7).

The aim of this study was to take a different approach in an effort to clarify the complex interrelationship that exists between obesity, IR, and ICR. Specifically, the majority of prior studies used linear associations to dissect the degree to which these three metabolic variables were related. We began our analysis by stratifying the experimental population into four dichotomous categories, varying in adiposity and insulin sensitivity. In addition, the insulin suppression test (IST) was used to obtain a direct measurement of insulin-mediated glucose disposal in all participants (8-11). Since octreotide is infused during the IST to suppress endogenous insulin secretion, this approach should also provide a more precise estimate of ICR.

Methods

Subjects

Experimental data were obtained from 126 males and 151 females in our ongoing registry who responded to newspaper advertisements describing studies related to the effects of IR on glucose and insulin metabolism. Subjects ranged from 19 to 69 years of age, had overweight or obesity according to body mass index ([BMI]: 25–40 kg/m²), and were of a non-Hispanic white race/ethnicity. Individuals were recruited between 2003 and 2013. Nondiabetic status of the subjects was determined based on the following criteria: no known medical history of diabetes, no use of medications known to affect carbohydrate metabolism, and fasting glucose levels < 126 mg/dL. All individuals also had no history of coronary artery, kidney, or liver disease, and all underwent the IST to quantify IR and ICR. All subjects provided written informed consent approved by the Stanford Institutional Review Board prior to participating in any experimental procedures.
TABLE 1 Anthropometric and biochemical characteristics of the study subjects stratified according to the insulin sensitivity and obesity

|               | Insulin sensitive |               | Insulin resistant |               |
|---------------|-------------------|---------------|-------------------|---------------|
|               | Non-obese | Obese | P-value<sup>a</sup> | Non-obese | Obese | P-value<sup>a</sup> |
| N             | 97        | 42    | 0.093             | 45        | 93    | 0.279             |
| SSPG (mg/dL)  | 95.6 ± 25.5 | 109.7 ± 26.6 | <0.001           | 206.5 ± 40.1 | 214.3 ± 39.4 | <0.001           |
| Age (years)   | 52.3 ± 7.9  | 53.3 ± 8.4  | 0.536             | 53.0 ± 10.0  | 52.6 ± 9.1   | 0.903             |
| Sex (men)     | 42 (43.3%) | 20 (47.6%) | 0.588             | 17 (37.8%)  | 47 (50.5%)  | 0.178             |
| BMI (kg/m<sup>2</sup>) | 27.5 ± 1.4 | 32.6 ± 1.8 | <0.001           | 27.8 ± 1.5  | 33.8 ± 2.6  | <0.001           |
| Waist circumference (cm) | 95.3 ± 6.6 | 109.0 ± 8.3 | <0.001           | 96.9 ± 6.5  | 109.6 ± 8.5 | <0.001           |
| ALT (IU/L)    | 29.4 ± 11.8  | 30.7 ± 11.9 | 0.499             | 30.3 ± 14.5  | 38.0 ± 17.7 | 0.019             |
| eGFR (mL/min/1.73 m<sup>2</sup>) | 95.3 ± 20.0 | 95.8 ± 21.7 | 0.971             | 103.1 ± 21.9 | 93.3 ± 24.0 | 0.072             |
| Fasting glucose (mg/dL) | 95.7 ± 10.1 | 97.6 ± 10.0 | 0.203             | 102.0 ± 10.1 | 101.6 ± 9.6 | 0.817             |
| Fasting insulin (μU/mL) | 8.27 ± 7.58 | 9.82 ± 5.83 | 0.036             | 14.0 ± 8.37  | 17.99 ± 9.46 | 0.016             |
| Insulin clearance (L/min/m<sup>2</sup>) | 0.498 ± 0.131 | 0.509 ± 0.136 | 0.865             | 0.445 ± 0.104 | 0.407 ± 0.156 | 0.145             |

Data are means ± standard deviations.

<sup>a</sup>P-values are for comparisons between the non-obese and obese groups.

SSPG, steady-state plasma glucose; BMI, body mass index; ALT, alanine aminotransferase; eGFR, estimated glomerular filtration rate.

Experimental end points

**Insulin suppression test (IST).** Whole body insulin action was directly measured using a modified version (8) of the IST (9); the values for IR obtained with this approach are highly correlated (r ~ 0.9) with those obtained using the hyperinsulinemic-euglycemic clamp technique (10,11). Briefly, after an overnight fast, an intravenous catheter was placed in each arm of the subjects; one catheter was used to administer a 180-min infusion of octreotide (0.27 μg/m<sup>2</sup>/min), insulin (32 μU/m<sup>2</sup>/min), and glucose (267 mg/m<sup>2</sup>/min); while the other catheter was used for the collection of blood samples. Blood samples were drawn at 10-min intervals during the last 30 min to measure the steady-state plasma glucose (SSPG) and steady-state plasma insulin (SSPI) concentrations. Since SSPI concentrations are similar in all subjects, the SSPG concentration provides a direct and specific measure of insulin-mediated glucose disposal; the higher the SSPG concentration, the greater the IR.

**Measurement of ICR.** ICR (units: L/min/m<sup>2</sup>) was estimated by dividing the insulin infusion rate by the SSPI concentration. Insulin determinations were made with the ultrasensitive insulin assay [Cat#33410] on the Access 2 immunoassay system (Beckman coulter), and had an inter-assay CV of 6.43 and an intra-assay CV of 5.61. The glomerular filtration rate (GFR) was calculated using the abbreviated Modification of Diet in Renal Disease formula: estimated GFR (eGFR) = 186.3 × SCR<sup>-1.154</sup> × age<sup>-0.203</sup> (or × 0.742 if female), where SCR is serum creatinine expressed in milligrams per deciliter.

**Definition of IR and obesity.** IR was defined as an SSPG concentration ≥150 mg/dL; a cut point shown in prospective studies to identify apparently healthy individuals who developed clinical syndromes related to IR (12,13). BMI was used to classify individuals as obese (BMI ≥30 kg/m<sup>2</sup>) or non-obese (BMI < 30 kg/m<sup>2</sup>). With these criteria, participants were placed into four experimental groups: non-obese/insulin sensitive; obese/insulin sensitive; non-obese/insulin resistant; and obese/insulin resistant.

**Statistical analysis.** All data are presented as means ± standard deviations (SD) unless stated otherwise. If necessary, a logarithmic transformation was performed to achieve a normal distribution. The Chi squared (χ<sup>2</sup>) and independent t-tests were used to compare the proportions and means, respectively, between the groups. Pearson’s correlation coefficients between ICR and experimental variables were calculated. Multiple linear regression models were used to identify factors associated with IR. Potential predictors of ICR evaluated were age, sex, BMI or waist circumference (WC), eGFR, alanine aminotransferase (ALT), and fasting plasma insulin. eGFR and ALT were added as surrogates of kidney and liver function, respectively, since both organs play vital roles in insulin clearance (14). All data were analyzed using the SPSS statistical package (SPSS, Chicago, IL). P-value < 0.05 was considered to indicate statistical significance and was not adjusted for multiple comparisons.

Results

Anthropometric and metabolic characteristics of the four experimental groups are presented in Table 1. By selection SSPG concentrations are increased approximately 2-fold in both of the insulin-resistant groups. However, SSPG concentrations do not vary as a function of differences in obesity in either the insulin-resistant or insulin-sensitive subgroups. Focusing initially on the insulin-sensitive groups, the subgroup with obesity had significantly higher values for BMI, WC, and fasting plasma insulin concentration. However, the values for ICR were essentially identical in the groups with and without obesity who were insulin sensitive. In the insulin-resistant groups, fasting insulin concentration was also higher in the obese subgroup but ICR was not different between the groups with and without obesity. ALT was significantly higher in the group with obesity.

Differences in the impact of obesity (BMI) vs. IR (SSPG concentration) on ICR and fasting plasma insulin concentration are illustrated in Figure 1. The results in Figure 1A compare the impact of obesity (BMI) and IR (SSPG concentration) on ICR. These data demonstrate...
that ICR values are lower in those who are insulin resistant, whether or not they have obesity, but there is no effect of obesity on ICR in either insulin-sensitive or insulin-resistant individuals. In contrast, Figure 1B demonstrates that fasting insulin concentrations are significantly higher in individuals with obesity, irrespective of their being insulin sensitive or insulin resistant.

Table 2 presents the univariate and multivariate relationships between ICR and possible modulators of its activity. At the univariate level, every factor other than age was significantly correlated with ICR. However, when adjusted for other relevant covariates, only the relationships between ICR and concentrations of both fasting plasma insulin and SSPG remained statistically significant. It should be emphasized that there was no significant relationship between BMI and ICR, and the same findings were seen when WC was substituted for BMI. We also conducted a simplified regression analysis with only SSPG and BMI as independent variables and ICR as the dependent variable. SSPG was significant (standardized beta coefficient $\beta = -0.437, P < 0.001$) while BMI remained insignificant ($\beta = -0.037, P = 0.543$).

### Discussion

At the simplest level, the results presented provide straight-forward answers to some of the issues raised in the Introduction. Thus, the results demonstrated that increases in SSPG concentration, a direct measure of IR at the whole body level, and increases in plasma insulin concentration were independently related to decreases in ICR. As such, our findings are consistent with previous descriptions of this relationship (2,15). A second major question addressed in this study was whether obesity was also independently related to increases in plasma insulin concentration and decreases in ICR. In this instance the findings are more complicated. Plasma insulin concentrations were significantly higher in individuals with obesity, whether they were insulin sensitive or insulin resistant (Fig. 1B). However, ICR did not vary as a function of obesity in either the insulin-resistant or insulin-sensitive subgroups (Fig. 1B), and results of the multiple linear regression analysis (Table 2) displays the lack of an independent relationship between adiposity and ICR.

The relationship among obesity, plasma insulin concentration, and ICR demonstrated in our study raises the following question: in the absence of an effect on ICR, why were plasma insulin concentrations elevated in individuals with obesity? As indicated earlier, there is evidence that obesity is associated with both an increase in insulin secretion and a decrease in ICR (1-7). Although a logical conclusion, hyperinsulinemia in obesity is conventionally assumed to result from increased insulin secretion in an attempt to compensate for obesity-associated IR (1-3), and we found plasma insulin concentrations to be higher in persons with obesity, whether they were insulin resistant or insulin sensitive. A possible solution to this conundrum can be found in the findings (1) of the European Group for the Study of Insulin Resistance (EGIR). In addition to describing an independent relationship between IR and insulin secretion, these authors found that degree of adiposity also had an independent relationship with increases in insulin secretion. Therefore, elevated plasma insulin concentration in obesity, irrespective of IR status and ICR, may relate to enhanced insulin secretion rate.

### Table 2 Univariate and multivariate linear regression analyses of insulin clearance with anthropometric and biochemical parameters

|                   | Univariate analysis |         | Multivariate analysis |         |
|-------------------|---------------------|---------|-----------------------|---------|
|                   | $\beta$              | $P$-value | $\beta$               | $P$-value |
| Age               | 0.016                | 0.793    | 0.019                 | 0.744    |
| Sex (women vs. men)| -0.177              | 0.003    | -0.133                | 0.059    |
| BMI               | -0.233              | <0.001   | -0.012                | 0.836    |
| ALT               | -0.142              | 0.020    | 0.033                 | 0.563    |
| eGFR              | 0.132               | 0.031    | 0.073                 | 0.270    |
| Fasting glucose   | -0.142              | 0.019    | 0.082                 | 0.171    |
| Fasting insulin   | -0.490              | <0.001   | -0.301                | <0.001   |
| SSPG              | -0.454              | <0.001   | -0.312                | <0.001   |

$\beta$, standardized beta coefficient; BMI, body mass index; ALT, alanine aminotransferase; eGFR, estimated glomerular filtration rate; SSPG, steady-state plasma glucose.
The second question relates to our inability to replicate the finding of other investigators that obesity, per se, was independently related to a decrease in ICR (4-7). Since these studies differed in overall protocol, we can only speculate upon the potential reasons for disparate findings. For example, Erdmann et al. (4) reported a weight-dependent decrease in ICR in 291 individuals, stratified into 5 BMI groups. In addition, IR as estimated by HOMA-IR was also greater with increasing BMI. Since the decrease in ICR was not analyzed in the context of the increase in IR, these results do not necessarily support the notion that obesity, per se, decreases ICR. Marini and colleagues (7) attempted to avoid the potentially confounding issue of IR by comparing ICR in 3 groups: non-obese/insulin sensitive; obese/insulin sensitive; and obese/insulin resistant. ICR was significantly decreased in only the obese/insulin-resistant group. These findings demonstrate that persons with obesity can differ in terms of both IR and ICR. However, the absence of a non-obese/insulin-resistant group does not seem to permit a definitive conclusion as to whether obesity or IR was responsible for the association with a decrease in ICR.

Dealing with the differences between our findings and those of the IRAS Family Study (5) and the IR Atherosclerosis Study (6) is more complicated. To begin with the methods of assessing IR (insulin suppression test vs. the frequently sampled intravenous glucose tolerance test, FSIVGTT) and insulin concentration (fasting plasma insulin vs. acute insulin response to intravenous glucose) were quite different. Perhaps of greater importance were differences in how ICR was calculated. Our calculations involved dividing the exogenous insulin infusion rate by the SSPI concentration during a constant infusion of glucose and insulin, while endogenous insulin was suppressed. In contrast, the IRAS investigators calculated ICR as the ratio of the dose of an acute injection of insulin over the incremental area under the curve of insulin from 20 min to infinity (5.6). ICR has been shown to be overestimated when insulin is given as a bolus compared with a constant infusion (16). In addition, impact of endogenous insulin secretion rate on ICR is ignored in most calculations, which may explain the greater variability in ICR with the FSIVGTT (5.6) as well as the clamp (7). It is obvious that we can only speculate as to how these differences might affect the measurement of ICR. Perhaps more to the point, although the IRAS investigators concluded that both obesity and IR were independent predictors of ICR in African Americans and Hispanics (5.6), only IR was an independent predictor in non-Hispanic whites (6). Thus, at least in the case of non-Hispanic whites, our findings and those of the IRAS investigators are in agreement; IR is an independent predictor of ICR, but obesity is not.

Although the results of our study seem straightforward, interpretation of our findings is limited to some extent by the nature of the experimental protocol. Most importantly, since we used a cross-sectional design, caution must be exercised in differentiating causal relationships from associations. In addition, participants were non-Hispanic whites, and the present findings cannot be extrapolated to other racial groups. In particular, our experimental protocol had certain strengths; including a relatively large number of participants, divided into 4 dichotomous groups, use of specific methods to quantify insulin sensitivity and insulin clearance, and the exclusion of individuals with conditions that could affect glucose metabolism.

In conclusion, our results add further evidence that differences in ICR play an important role in regulation of plasma insulin concentration (1-7). In addition, they provide additional support for the notion that decreases in ICR are independently associated with magnitude of IR, not excess adiposity, in non-Hispanic whites (1.6). However, at least three major questions remain concerning the factors that modulate plasma insulin concentration: (1) is the independent relationship between excess adiposity and decreased ICR in African Americans and Hispanics (5.6) a function of race or methodology; (2) what is responsible for the finding that IR cannot account for the increased insulin secretion and hyperinsulinemia in non-Hispanic white individuals with obesity (1); and (3) what is the pathophysiologic explanation for the association between IR and ICR. Obviously, answers to these questions will go a long way in our understanding of the association between obesity and hyperinsulinemia—the issue addressed in this study.

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