Metabolic Changes Following Sibutramine-Assisted Weight Loss in Obese Individuals: Role of Plasma Free Fatty Acids in the Insulin Resistance of Obesity

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The relationship between insulin-mediated glucose disposal and daylong free fatty acid (FFA) concentrations before and after sibutramine-assisted weight loss was investigated in 24 healthy, normotensive, nondiabetic, obese women (body mass index [BMI] >30.0 kg/m^2). The 24 volunteers were defined as being insulin-resistant (IR) or insulin-sensitive (IS) on the basis of their steady-state plasma glucose (SSPG) concentration in response to a 180-minute continuous intravenous infusion of octreotide, insulin, and glucose. The mean (± SEM) SSPG concentrations were significantly higher ($P < .001$) in the IR group (219 ± 7 *v* 69 ± 6 mg/dL) at baseline. The IR group also had significantly higher plasma glucose ($P = .002$), insulin ($P < .001$), and FFA ($P = .02$) concentrations measured at hourly intervals from 8 AM to 4 PM, before and after breakfast (8 AM) and lunch (noon). Weight loss in response to an energy-restricted diet for 4 months and sibutramine (15 mg/d) was comparable in the 2 experimental groups (8.6 ± 1.3 *v* 7.9 ± 1.4 kg). SSPG concentrations decreased significantly ($P < .001$) following weight loss (219 ± 7 to 144 ± mg/dL) in the IR group, but there was no change in the SSPG of the IS group (69 ± 6 to 73 ± 7 mg/dL. The improvement in insulin sensitivity in the IR group after weight loss was associated with a significant decline in daylong plasma glucose ($P > .001$) and insulin ($P = .02$) concentrations, without a weight-loss–associated decrease in daylong plasma FFA responses. In contrast, there was no significant change in plasma glucose, insulin, and FFA concentrations following weight loss in the IS group. These results indicate that daylong FFA concentrations vary substantially in obese individuals as a function of whether they are IR or IS. Furthermore the observation that the IR group was more insulin-sensitive after weight loss, associated with lower daylong insulin concentrations in the absence of a significant decrease in circulating FFA concentrations, suggests that resistance to insulin-mediated glucose disposal in obese individuals cannot be entirely due to high FFA levels.

ALTHOUGH OBESITY AND resistance to insulin-mediated glucose disposal have been known to be associated for many years,[1-3] the physiologic basis of this relationship is not well understood. In that context, we have previously published evidence that daylong plasma free fatty acid (FFA) concentrations are elevated in obese individuals.[4] These results led us to speculate that the resistance to insulin-mediated glucose disposal associated with obesity might be secondary to the increased plasma FFA concentration.[5-8] However, it has become apparent that not all obese individuals are insulin-resistant (IR)[9-11]. Because we did not quantify insulin-mediated glucose disposal in the obese patients in our previous study,[4] it is not clear if elevated plasma FFA concentrations occur in all overweight individuals or only in those who are also IR. If elevated plasma FFA concentrations are simply the result of increased adipose tissue, the values should be similar in all obese individuals and not vary as a function of differences in insulin-mediated glucose disposal. Alternatively, if increased plasma FFA concentrations are a consequence of adipocyte insulin resistance in obese individuals, then FFA concentrations should be higher in IR as compared with equally obese, insulin-sensitive (IS) individuals. This study was performed to evaluate these two hypotheses.

MATERIALS AND METHODS

The study population consisted of 24 obese, women volunteers from the San Francisco Bay area who had responded to advertisements placed in local newspapers. Participants were required to have a body mass index (BMI) between 30 and 36 kg/m^2 and nondiabetic according to the criteria of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus.[12] Exclusions included psychiatric instability, history of gastrointestinal surgery for weight reduction, recent change in exercise pattern, history of hypertension, cardiac arrhythmia, coronary artery disease, seizures, use of monoamine-oxidase inhibitors, and pregnancy. Potential subjects (n = 44) who met these general criteria were further screened at the General Clinical Research Center (GCRC) at Stanford Medical Center. Weight was measured with subjects wearing light clothing. BMI was calculated as weight (kilograms) divided by height (square meters). The study was approved by the Stanford University Human Subjects Committee, and all participants gave informed, written consent.

To create an IS and an IR group, insulin-mediated glucose disposal was quantified in those who met the general inclusion criteria by a modification[13] of the insulin suppression test as originally described.[14] Briefly, subjects were infused for 180 minutes with octreotide (0.27/m^2 · min), insulin (25 mU/m^2 · min), and glucose (240 mg/m^2 · min). Blood was drawn at 10-minute intervals from 150 to 180 minutes of the infusion to measure plasma glucose[15] and insulin[16] concentrations, and the mean of these 4 values used as the steady-state plasma insulin (SSPI) and glucose (SSPG) concentrations for each individual. As SSPI concentrations were similar in all subjects, the SSPG concentration provided a direct measure of the ability of insulin to mediate disposal of an infused glucose load; the higher the SSPG concentration, the more insulin-resistant the individual.

Insulin resistance was defined as a SSPG value greater than 160 mg/dL and insulin sensitivity as a SSPG concentration less than 100 mg/dL. These values represent cut-points for separating the upper and lower 40% of SSPG concentrations as measured in 490 healthy volun-

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teers.[17] Of the 44 volunteers who met the general criteria, 13 IR and 11 IS individuals began and completed the 4-month weight loss period. The proportion of individuals with normal fasting or impaired fasting glucose was similar in the IR (9/4) and IS (8/3) groups.

Before the period of weight loss, blood was drawn after an overnight fast for determination of fasting plasma glucose,[15] insulin,[16] and FFA[18] concentrations. Dinner the evening prior to any testing was standardized to comprise 40% of estimated daily caloric requirements, containing as percent of total calories, 15% protein, 43% carbohydrate, and 42% fat. In addition to fasting values, plasma glucose, insulin, and FFA concentrations were measured hourly for 8 hours following 2 standard test meals, each containing as percent of total calories, 15% protein, 43% carbohydrate, and 42% fat. Meals were given at 8 AM and 12 PM, with breakfast comprising 20%, and lunch 40% of estimated daily caloric requirements.

Following these baseline measurements, subjects were placed on a hypocaloric diet and given sibutramine, 15 mg/day. The sibutramine was provided by Knoll Pharmaceuticals (Mount Olive, NJ) as part of their partial financial support of this study, but they were not involved in either the data analysis or the preparation of this report. The Harris-Benedict equation[19] was used to estimate each volunteer's basal energy expenditure, and an activity-factor was added to estimate total caloric requirement (basal energy expenditure $\times$ 1.3 to 1.5). Daily caloric intake for each subject during the study was their estimated total caloric intake, minus 500 kcal, which over a 1-week period should lead to weight loss of approximately 1 lb. Body weight was determined with the subject wearing light clothing at baseline and weekly thereafter. The weight loss period was 4 months in duration, after which time subjects were instructed on a weight maintenance diet, which they continued for a minimum of 14 days. During this period, weight fluctuated by less than 1.0 kg in any individual. They were then readmitted to the GCRC, and all the baseline measures repeated. Standardized test meals during metabolic testing after weight loss were eucaloric as estimated by the Harris-Benedict equation for each subject's final weight.

Data are expressed as the mean $\pm$ SEM, and all values were normally distributed. Student's unpaired *t* test was used for comparisons involving age, weight, BMI, and SSPG. Two-way analysis of variance (ANOVA) was used for comparisons involving multiple time points, including daylong plasma glucose, insulin, and FFA concentrations. Comparisons were made between IR and IS subjects at baseline and after weight loss. In addition, comparisons were made between baseline and postweight loss values for each group. Spearman's correlation coefficients were used to compare absolute and percent change in FFA [(postweight loss-baseline value)/baseline value] with absolute and percent change in insulin resistance.

RESULTS

Of the 44 qualifying subjects, 20 did not complete the study. Three subjects never began the diet, 9 dropped out during the first month, 4 during the second month, and 4 during the third or fourth months. Reasons for dropout during the first month

Table 1. Mean (SEM) Baseline Demographic Characteristics of the Two Groups

Variable	IR (n=13)	IS (n=11)
SSPG (mg/dL)	219 $\pm$ 7	69 $\pm$ 6
Age (yr)	48 $\pm$ 3	45 $\pm$ 2
Weight (kg)	84.1 $\pm$ 1.9	86.4 $\pm$ 2.2
BMI (kg/m^2)	31.8 $\pm$ 0.6	30.9 $\pm$ 0.4

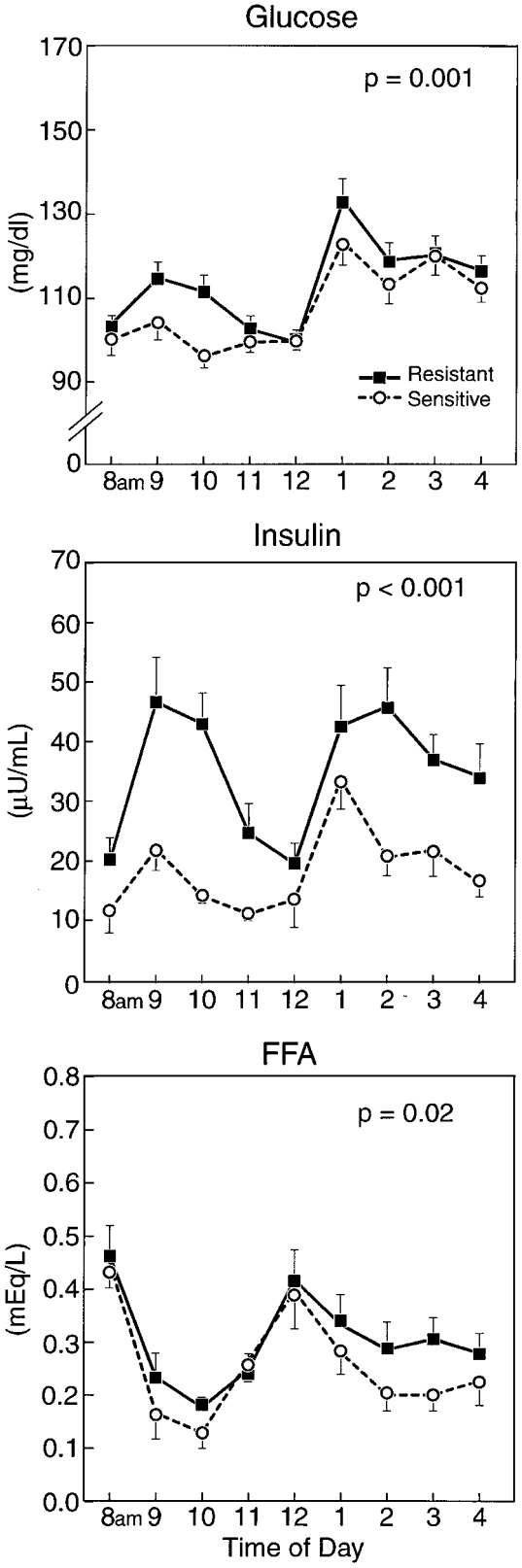

Fig 1. Comparison between the plasma glucose, insulin, and FFA concentrations in the IR *v* the IS groups before weight loss. Measurements were made at hourly intervals from 8 AM to 4 PM, before and after breakfast (8 AM and lunch (noon).

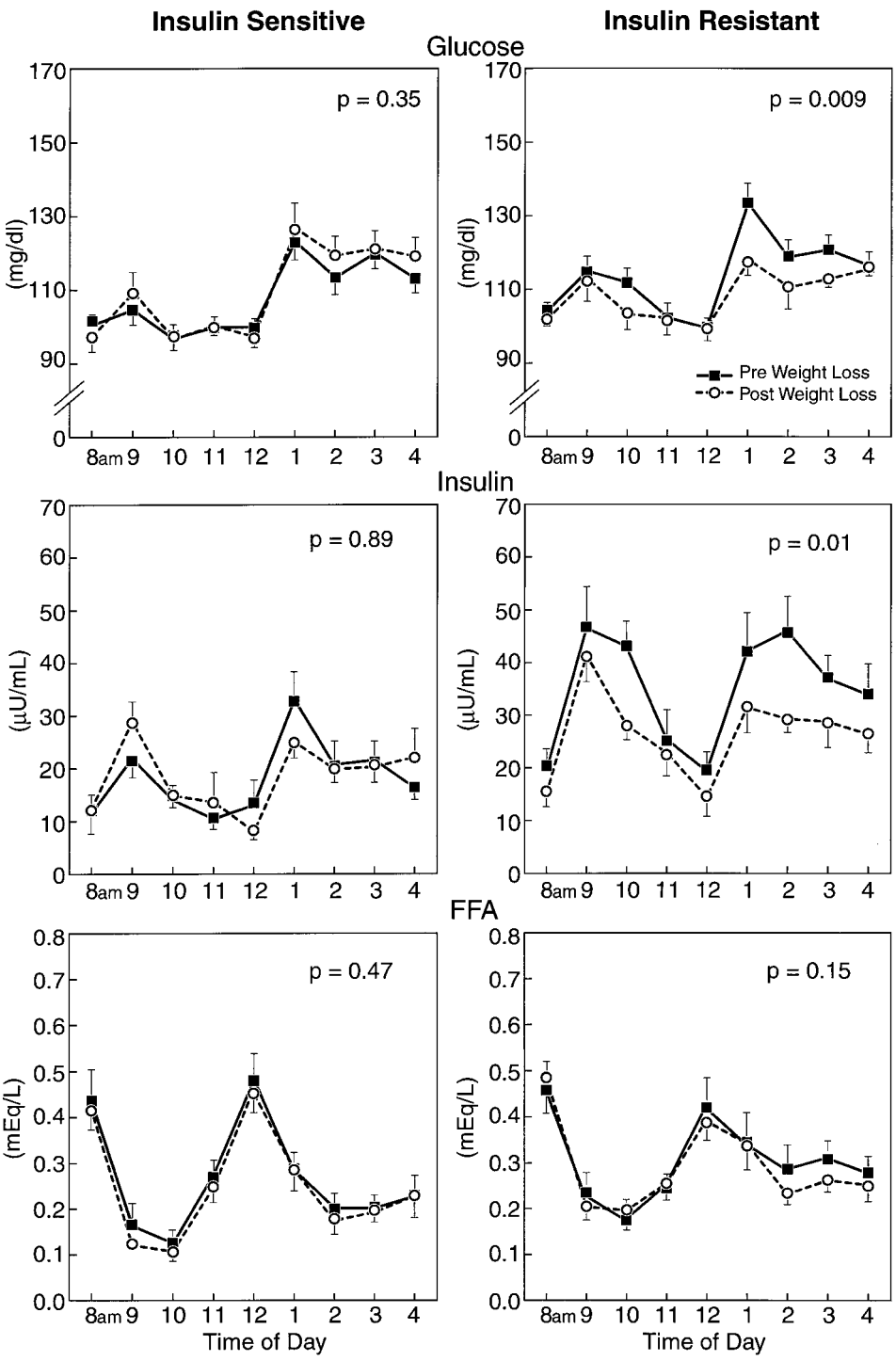

Fig 2. Effect of weight loss on daylong plasma glucose insulin, and FFA concentrations in the IR *v* the IS groups. Measurements were made at hourly intervals from 8 AM to 4 PM, before and after breakfast (8 AM) and lunch (noon).

included starting antidepressant medication (n = 1), geographic relocation (n = 1), "too busy" (n = 2), and unknown (n = 4). Reasons for dropout during the second month included stopping sibutramine (n = 1), lightheadedness (n = 1), travel (n = 1), and unknown (n = 1). During the third and fourth months, subjects dropped out due to stopping sibutramine (n = 1), unable to comply with dietary restriction (n = 1), and unknown (n = 2). Twenty-four subjects completed the study, of whom 11 were IS and 13 were IR. Drop outs did not differ from completers with regard to age, BMI, baseline weight, blood pressure, or SSPG concentrations.

Table 1 lists the baseline demographic characteristics of the IR and IS groups. The SSPG concentrations were significantly higher in the IR group by selection, but all other variables, including weights, were similar in the 2 groups. However, as shown in Fig 1, the IR group had significantly higher daylong

plasma glucose ($P = .002$), insulin ($P < .001$), and FFA ($P = .02$) concentrations before starting the calorie-restricted diet.

The amount of weight loss in response to the calorie-restricted diet was similar in the 2 groups (8.6 ± 1.3 kg *v* 7.9 ± 1.4kg in the IR and IS groups, respectively). In contrast the effect of weight loss on SSPG concentrations was quite different in the 2 groups. The SSPG concentrations decreased significantly ($P < .001$) in association with weight loss in the IR group from 219 ± 7 mg/dL to 144 ± 14 mg/dL, whereas there was no significant change in SSPG concentrations in the IS group (69 ± 6 mg/dL before and 73 ± 7 mg/dL after weight loss).

Daylong plasma glucose, insulin, and FFA concentrations before and after weight loss are shown in Fig 2. The left panel of Fig 2 displays the results in the IS group and indicate that the values before and after weight loss were essentially identical for all variables. In contrast, results in the right panel of Fig 2 demonstrate that weight loss in the IR group was associated with significantly lower daylong concentrations of plasma glucose ($P < .001$) and insulin ($P < .02$). However, there was no significant difference between the daylong FFA concentrations before and after weight loss in the IR group. The observation that plasma FFA concentrations did not change in either group with weight loss was consistent with the fact that the decrease in plasma FFA concentrations of the 2 groups was quite similar. More specifically, the total integrated FFA response decreased by 7% and 10% in the IS and IR groups, respectively.

Comparisons of the plasma glucose, insulin, and FFA concentrations in the 2 groups after weight loss are shown in Fig 3. It is apparent that the daylong plasma glucose concentrations of the 2 groups were no longer different after weight loss, whereas the IR group still had higher daylong plasma insulin ($P < .001$) and FFA ($P < .05$) concentrations.

To see if there was a relationship between postweight loss changes in daylong FFA concentrations and improvement in SSPG concentrations, correlation coefficients were calculated between both the absolute and percent decreases in FFA response and those in SSPG concentration. These calculations were performed in each group by itself, as well as in the whole population, and none of these relationships were significant (correlations coefficients varied from $r = 0.09$ to $r = 0.35$).

DISCUSSION

The results presented have provided straightforward answers to the issues raised earlier in our report. In the first place, the results show that daylong FFA concentrations are not similar in all obese individuals, but are significantly higher in those who are IR as compared with individuals who are equally obese, but IS. As such, these data demonstrate that circulating plasma FFA concentrations in obese individuals are not a simple reflection of adiposity, but vary considerably as a function of whether the obese individuals are IR or IS.

Second, whereas SSPG concentrations decreased significantly following weight loss in the IR group, daylong plasma FFA concentrations were not significantly lower. Furthermore, there was no relationship between the decrease in daylong plasma FFA concentrations following weight loss and decrease in SSPG concentrations, and this was true for the entire popu-

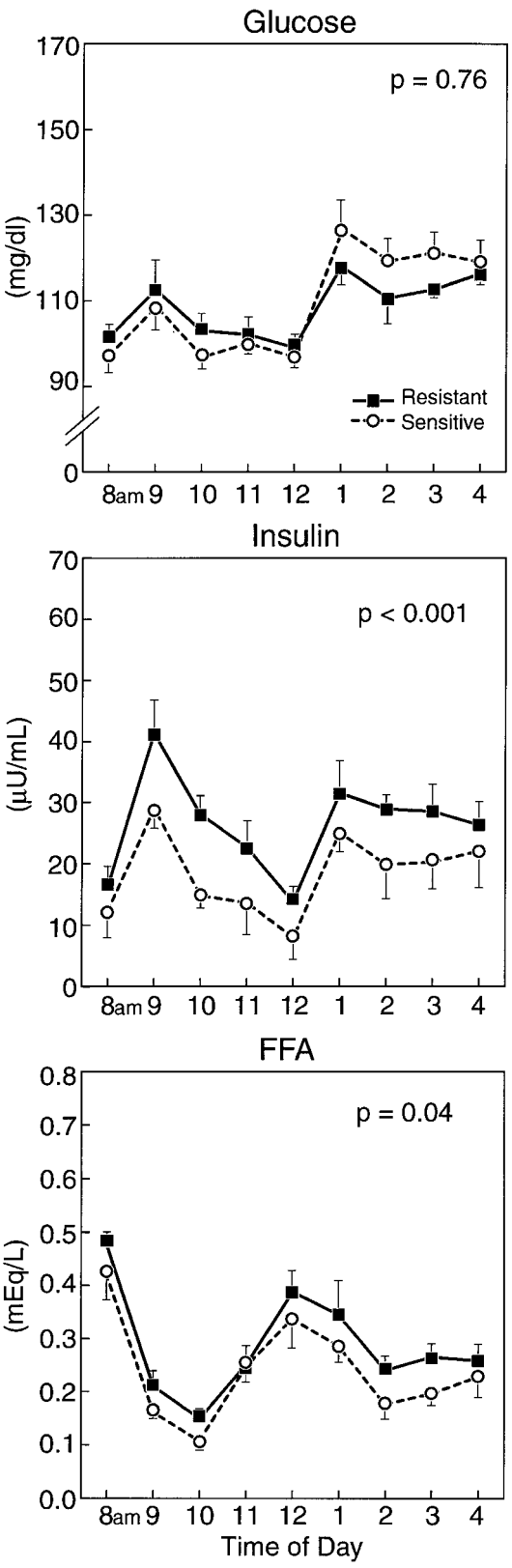

Fig 3. Comparison between plasma glucose, insulin, and FFA concentrations in the IR and the IS groups following weight lost. Measurements were made at hourly intervals from 8 AM to 4 PM, before and after breakfast (8 AM and lunch (noon).

lation, as well as when each group was analyzed separately. Thus, it seems difficult to argue that the improvement in insulin-mediated glucose disposal with weight loss in the IR group was a simple matter of lower daylong plasma FFA concentrations.

Although the current study demonstrates the fact that insulin resistance in obese individuals is accompanied by elevated daylong FFA concentrations, our goal was not to define the causal relationship between these 2 variables. On the other hand, the results provide information that is relevant to this issue. The association between higher concentrations of SSPG and daylong FFAs can be interpreted in 2 ways: the higher FFA concentrations could be the cause of the insulin resistance,[5-8] or the increased FFA concentration could be the consequence of excessive lipolysis, secondary to adipose tissue insulin resistance.[20-22] Our ability to discern between these 2 alternatives is confounded by the fact that relatively small changes in insulin concentration have relatively major effects on FFA concentrations.[23] However, given these limitations, it is possible to offer a coherent argument, based on the changes following weight loss, that increased FFA concentrations in the IR group are secondary to adipose tissue insulin resistance and not the cause of the higher SSPG concentrations.

Perhaps the most compelling argument in support of the view that elevated FFA concentrations cannot be solely responsible for muscle insulin resistance comes from consideration of the changes in SSPG and plasma FFA concentrations in the 2 groups associated with weight loss. Specifically, daylong plasma FFA concentrations fell to a similar degree with weight loss in the IR and IS groups. On the other hand, SSPG concentration decreased from 219 $\pm$ 7 to 144 $\pm$ 14 mg/dL ($P <$ .001) following weight loss in the IR group, whereas there was no change in the IS group. Essentially the same point can be made by focusing on the daylong plasma insulin responses. Thus, despite comparable decreases in day-long FFA concentrations with weight loss in both groups, the daylong insulin response only was lowered in the IR group.

Parenthetically, the relationship between plasma insulin and FFA responses described above is of some relevance to the notion[24] that hyperinsulinemia in obese, IR individuals results from a decrease in hepatic removal of insulin, secondary to increased FFA concentrations.[25,26] If that were the case, it is difficult to understand why daylong plasma insulin concentrations fell significantly in association with weight loss in the IR group in the absence of any change in daylong plasma FFA concentrations.

Sibutramine was used in this study in an effort to enhance the weight loss in response to a calorie-restricted diet under conditions in which dietary intervention was limited menu suggestions. Based on published data, the use of sibutramine should not have been a confounding variable. For example, to the best of our knowledge, there are no published reports of an effect of sibutramine on either insulin resistance or plasma FFA or insulin concentrations. Sibutramine-assisted weight loss of 5% of body weight has been associated with decreases in plasma triglyceride concentrations,[27] but a similar effect has been described with similar weight loss in the absence of sibutramine.[28] Improvement in plasma glucose concentrations have only been observed with a greater decrease (>15%) in body weight.[27] Thus, there is no reason to believe that the differences in the behavior of the IR and IS groups were related to the use of sibutramine.

In conclusion, the results of this study have shown that daylong plasma FFA concentrations are not similar in all obese individuals, but are significantly different in equally obese individuals as a function of whether they are IR or IS. Further, insulin resistance and compensatory hyperinsulinemia in obese individuals decrease significantly after moderate weight loss, without a significant change in FFA concentrations. These results do not imply that variations in plasma FFA concentrations are without a role in regulation of insulin action on muscle, and because plasma FFA concentrations are not necessarily an accurate measure of lipolysis, our findings do not provide a precise estimate of the effects of obesity and/or insulin resistance on the lipolytic response of adipose tissue. On the other hand, our results provide evidence that resistance to insulin-mediated glucose disposal in obese individuals is, at least to some extent, independent of elevated plasma FFA concentrations.

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