PRELIMINARY REPORT

Insulin Regulation of Plasma Free Fatty Acid Concentrations Is Abnormal in Healthy Subjects With Muscle Insulin Resistance

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This study evaluated the ability of insulin to regulate free fatty acid (FFA) concentrations in healthy nondiabetic subjects selected to be either insulin-resistant or -sensitive on the basis of insulin-mediated glucose disposal by muscle. Comparisons of steady-state plasma glucose (SSPG), insulin (SSPI), and FFA concentrations were made at the end of 3 infusion periods: (1) under basal insulin conditions (~10 μU/mL), (2) in response to isoproterenol-induced stimulation of lipolysis at the same basal insulin concentration, and (3) following inhibition of isoproterenol-induced lipolysis by a 2-fold increase in the insulin concentration. The results showed that steady-state FFA concentrations were significantly higher under basal conditions (360 ± 73 *v* 158 ± 36 μEq/L, $P = .02$), in response to isoproterenol-induced lipolysis (809 ± 92 *v* 433 ± 65 μEq/L, $P = .005$), and following insulin inhibition of isoproterenol-induced lipolysis (309 ± 65 *v* 159 ± 37 μEq/L, $P = .06$). These differences were found despite the fact that SSPG concentrations were also higher in insulin-resistant individuals during all 3 infusion periods. These results demonstrate that the ability of insulin to regulate plasma FFA concentrations is impaired in healthy subjects with muscle insulin resistance, indicating that insulin-resistant individuals share defects in the ability of insulin to stimulate muscle glucose disposal and to inhibit adipose tissue lipolysis.

THE ABILITY of physiological hyperinsulinemia to stimulate muscle glucose disposal is usually measured under steady-state conditions in response to a continuous infusion of insulin and glucose.[1,2] In this situation, the phrase "insulin resistance" refers to a relative reduction in the ability of a given increase in plasma insulin to stimulate glucose disposal by muscle during such infusion studies. Abnormalities in the ability of insulin to regulate plasma free fatty acid (FFA) concentrations are difficult to demonstrate under similar conditions, related to the sensitivity of adipose tissue to the antilipolytic effects of insulin.[3] However, evidence has been published indicating that the ability of insulin to suppress FFA concentrations is decreased in obese subjects and patients with type 2 diabetes.[3-6] On the other hand, relatively little is known about defects in the ability of insulin to suppress plasma FFA concentrations in healthy non-obese subjects, as compared with our knowledge of insulin-mediated glucose disposal in this same population.[7,8]

The present study was initiated to test the hypothesis that abnormalities in insulin regulation of plasma FFA concentrations are present in healthy non-obese subjects with muscle insulin resistance. To test this hypothesis, we selected 9 insulin-sensitive and 9 insulin-resistant individuals and compared their steady-state plasma FFA concentrations in response to a variety of physiological interventions.

SUBJECTS AND METHODS

Eighteen healthy individuals were recruited for these studies, defined as either insulin-sensitive (n = 9) or insulin-resistant (n = 9) as described subsequently. The 2 groups were similar in terms of age (54 ± 4 *v* 56 ± 3 years, gender (4 males and 5 females), and body mass index (25.0 ± 0.5 *v* 25.3 ± 0.9 kg/m^2). All participants were in good health with a normal medical history and physical examination, normal values on a routine hematological survey and chemical screening battery, and normal glucose tolerance on the basis of the fasting plasma glucose concentration.[9]

After providing written informed consent, the participants were admitted to the General Clinical Research Center on 2 occasions after an overnight fast for the following tests. Firstly, insulin-mediated glucose disposal by muscle was evaluated by a modification[10] of the insulin suppression test (IST) as initially described by our laboratory.[11] Briefly, an intravenous (IV) catheter was placed in each of the patient's arms. Blood was sampled from 1 arm for measurement of plasma glucose,[12] insulin,[13] and FFA[14] concentrations, and the contralateral arm was used for administration of test substances. Octreotide acetate (Sandostatin; Sandoz Pharmaceuticals, East Hanover, NJ) was administered at a rate of 0.27 μg/m^2/min to suppress endogenous insulin secretion. Simultaneously, insulin and glucose were infused at rates of 32 mU/m^2/min and 267 mg/m^2/min, respectively. Blood was sampled every 30 minutes until 150 minutes into the study, and then every 10 minutes until 180 minutes had elapsed. The 4 values obtained from 150 to 180 minutes were averaged and considered to represent the steady-state plasma glucose (SSPG) and insulin (SSPI) concentrations achieved during the infusion. Since SSPI concentrations are comparable in all individuals (~60 μU/mL), SSPG concentrations provide a direct estimate of insulin-mediated glucose disposal in each individual. The higher the SSPG, the more muscle insulin-resistant the individual. The mean SSPG concentration in the insulin-resistant group was 200 ± 14 mg/dL (range, 157 to 267), in contrast to a value of 80 ± 7 mg/dL (range, 46 to 121) in the insulin-sensitive individuals. The insulin-resistant and insulin-sensitive groups had essentially identical values for both plasma glucose (88 ± 8 *v* 87 ± 4 mg/dL, $P = .89$) and FFA (531 ± 59 *v* 469 ± 46, $P = .42$) on blood drawn before the IST began, whereas plasma insulin was significantly higher (14 ± 2 *v* 8 ± 1 μU/mL, $P = .01$) in the insulin-resistant individuals.

On a separate day, insulin's ability to regulate plasma FFA concentrations was assessed during a 260-minute infusion study. As for the IST, catheters were placed in each of the patient's arms and octreotide acetate was infused to suppress endogenous insulin secretion. To simulate basal conditions, insulin and glucose were infused at rates of 6 mU/m^2/min and 50 mg/m^2/min, respectively. Blood was drawn for measurement of plasma glucose, insulin, and FFA concentrations every

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30 minutes until 100 minutes into the study, and then every 10 minutes until 120 minutes had elapsed. The 3 values obtained from 100 to 200 minutes were averaged to represent the basal SSPG, SSPI, and FFA concentrations. At 120 minutes, isoproterenol was infused at a rate of 6.67 ng/kg/min to induce lipolysis, while the infusion rates were maintained. Blood was sampled every 10 minutes from 160 to 180 minutes to obtain SSPG, SSPI, and FFA concentrations in response to the isoproterenol infusion. At 180 minutes, the insulin infusion rate was increased to 15 mU/m^2/min to achieve an approximate doubling of the plasma insulin concentration, the glucose infusion rate was increased to 100 mg/m^2/min, and the isoproterenol infusion rate was unchanged. Blood was sampled every 10 minutes from 240 to 260 minutes to obtain SSPG, SSPI, and FFA concentrations.

Data are expressed as the mean ±SEM and were analyzed using the Systat 7.0.1 package (SPSS, Chicago, IL). The insulin-resistant and -sensitive groups were compared with Student's unpaired 2-tailed *t* test.

RESULTS

The top panel in Fig 1 presents SSPG, SSPI, and FFA concentrations in response to the basal insulin infusion in the 2 groups of subjects. Plasma insulin concentrations were similar

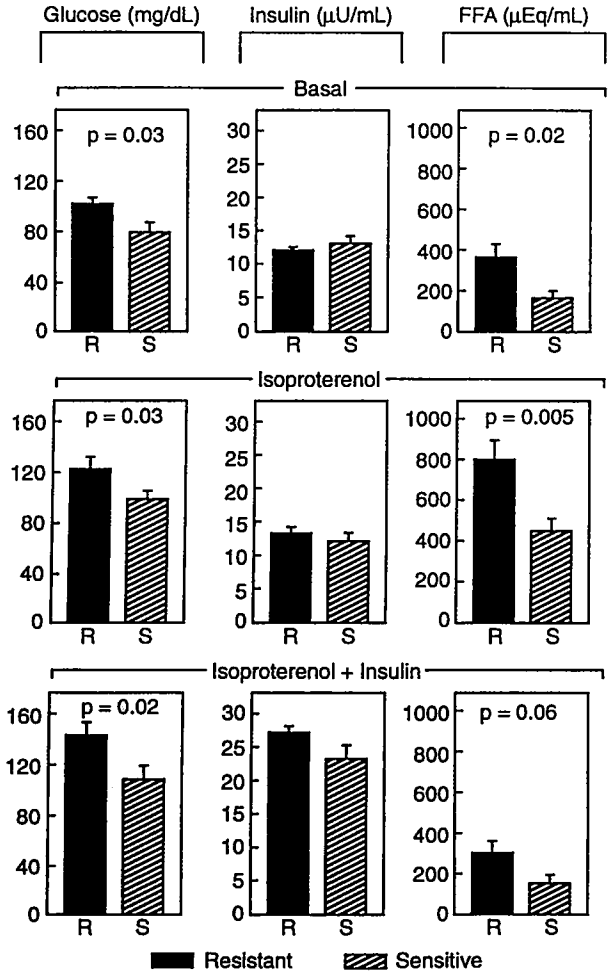

Fig 1. Steady-state plasma glucose, insulin, and FFA concentrations at the end of each infusion period under basal conditions, in response to isoproterenol-induced lipolysis, and following insulin inhibition of isoproterenol-induced lipolysis.

in the 2 groups, whereas plasma glucose under these conditions was higher in the insulin-resistant group (101 ± 5 *v* 79 ± 8, $P = .03$). At the same time, steady-state plasma FFA concentrations were significantly ($P = .02$) higher in the insulin-resistant group (360 ± 73 *v* 158 ± 36 μEq/L).

The steady-state responses when isoproterenol was added to the infusion are shown in the middle panel of Fig 1. In this situation, SSPG concentrations were also higher (124 ± 9 *v* 99 ± 6 mg/dL, $P = .03$) in the insulin-resistant individuals. Plasma insulin concentrations did not change in either group following the addition of isoproterenol to the infusion. Although steady-state plasma FFA concentrations increased in both groups in response to isoproterenol, the steady-state concentrations were much higher ($P = .005$) in the insulin-resistant group (809 ± 92 μEq/L) compared with the insulin-sensitive group (443 ± 65 μEq/L).

The ability of an approximate doubling of the SSPI concentration to suppress isoproterenol-induced lipolysis is shown in the bottom panel of Fig 1. The data in the middle panel demonstrate that plasma insulin concentrations were similar in the 2 groups, whereas both plasma glucose (149 ± 10 *v* 112 ± 11, $P = .02$) and FFA (309 ± 65 *v* 159 ± 37, $P = .06$) were higher in the insulin-resistant individuals.

DISCUSSION

By selecting 2 dichotomous groups with regard to the ability of steady-state physiological hyperinsulinemia to stimulate muscle glucose disposal, we have been able to clearly document differences in insulin's ability to regulate plasma FFA concentrations in non-obese healthy subjects. Individuals who were selected because their muscles were relatively resistant to the effects of physiological hyperinsulinemia on glucose disposal also demonstrated abnormalities in the ability of insulin to regulate plasma FFA concentrations. More specifically, steady-state plasma FFA concentrations were significantly higher in the insulin-resistant group when measured at basal insulin, in response to stimulation of adipose tissue lipolysis by isoproterenol, and following insulin inhibition of isoproterenol-stimulated lipolysis. In other words, individuals who are resistant to insulin's action on muscle are also unable to maintain plasma FFA concentrations in a normal manner. These findings are consistent with previous results from our research group.[15] However, the conclusions in our earlier study relied on the identification of statistically significant correlation coefficients within a population in which the relevant variables were distributed continuously, with reliance on our ability to adjust for relevant covariates. In the present instance, we have created 2 distinct groups, with the only apparent difference at the outset being their degree of muscle insulin resistance. We have thus avoided the reliance on statistical adjustments, and can directly compare the ability of insulin to regulate plasma FFA concentrations in groups dichotomous for muscle insulin resistance.

In addition to the differences in plasma FFA concentrations, plasma glucose concentrations were also higher during every infusion period in the insulin-resistant group. One obvious explanation for this difference during the first 2 infusion periods

is that the higher plasma FFA concentrations led to a decrease in muscle glucose uptake in the insulin-resistant individuals at basal insulin concentrations.[16] However, both glucose and insulin infusion rates were increased during the last infusion periods, and the higher plasma glucose concentrations in this instance might be related to the combined effect of higher FFA concentrations and the defect in insulin-stimulated glucose disposal by muscle in the insulin-resistant subjects.

The simplest explanation for the results shown in Fig 1 is that comparable defects exist within a given individual as regards the ability of insulin both to stimulate glucose uptake and to suppress adipose tissue lipolysis. This conclusion is persuasive concerning insulin regulation of plasma FFA concentrations under basal conditions, and is consistent with previous results showing that insulin inhibition of plasma FFA concentrations is decreased in association with obesity and type 2 diabetes.[3-6] Although the higher plasma FFA concentrations in response to isoproterenol suggest that muscle insulin resistance is associated with enhanced catecholamine-induced lipolysis, this finding appears to be in conflict with the results from Connacher et al[17] showing that the increase in FFA flux in response to an infusion of adrenaline was reduced in obese compared with lean individuals. However, obese individuals also had significantly higher plasma glucose and insulin concentrations during the adrenaline infusion. Given the sensitivity of adipose tissue to the antilipolytic effect of insulin[3] and the evidence that hyperglycemia per se is antilipolytic,[18] an attenuated effect of adrenaline to increase FFA flux in obese subjects[17] may not be unexpected.

The antilipolytic effects of an increase in plasma glucose and insulin also might have contributed to our observation that the difference in plasma FFA concentrations between the 2 groups following insulin suppression of isoproterenol-induced lipolysis was of marginal statistical significance ($P = .06$). Specifically, plasma glucose was significantly higher during this infusion period in the insulin-resistant subjects, and their plasma insulin concentration was also somewhat higher ($P = .19$). It is certainly possible that these differences could have falsely accentuated the decrease in steady-state plasma FFAs in the insulin-resistant group. Finally, the decision to increase the plasma insulin concentration 2-fold was arbitrary, and the differences in steady-state plasma FFA concentrations might have reached the level of statistical significance if another infusion rate were chosen.

In conclusion, the present results provide strong evidence that the ability of insulin to normally regulate plasma FFA concentrations is impaired in healthy subjects with muscle insulin resistance. The simplest explanation for these findings is that insulin-resistant individuals share defects in the ability of insulin to stimulate muscle glucose disposal and to inhibit adipose tissue lipolysis. On the other hand, measurement of plasma FFA concentrations only provides a surrogate estimate of lipolysis. In general, plasma FFA concentrations and total body FFA turnover are highly correlated,[19] but there are certainly exceptions to this generalization. For example, Wolfe and Peters[20] have shown that reesterification of FFA within adipose tissue is significantly increased during infusions of glucose and insulin. As such, the decrease in plasma FFAs is less useful as a marker of lipolysis under these conditions. On the other hand, this phenomenon was observed at higher glucose and insulin infusion rates and higher plasma glucose and insulin concentrations than in the current study. Consequently, it is likely, under the conditions of our protocol, that plasma FFA concentrations provide a reasonable estimate of adipose tissue lipolysis. In any event, the goal of our study was to test the hypothesis that the ability of insulin to regulate plasma FFA concentrations would be decreased in healthy subjects with muscle insulin resistance. Determining whether this phenomenon is due to a defect in antilipolysis and/or reesterification was not the aim of our study.

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