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Insulin dose response analysis of free fatty acid kinetics

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Abstract

Insulin regulation of free fatty acid (FFA) release is an important aspect of metabolic function; however, FFA release is exquisitely sensitive to insulin, which complicates the design and analysis of dose response experiments. We measured FFA ([^{3}H]palmitate) and glucose ([^{3}H]glucose) kinetics in 7 nonobese men, 7 nonobese women, 7 obese men, and 7 obese women by using a two-step insulin clamp (0.25 and 2.5 mU/kg fat-free mass per minute). Obese men and women were characterized as having a BMI of 28 or greater and body fat of 28% and 40% or greater for men and for women, respectively. Nonobese men and women had 22% and 35% or less body fat, respectively. All volunteers were Caucasian. Glucose disposal increased in a linear fashion with plasma insulin concentrations. The nonlinear suppression of plasma palmitate flux and concentrations could be linearized by logarithmically transforming both the insulin concentration and palmitate axes, except in nonobese men. We repeated the studies in 7 nonobese and 7 obese men, using 1.0 mU/kg fat-free mass per minute as the second insulin dose, which linearized the log-transformed lipolysis measures. The indices of insulin regulation of lipolysis predicted using 2 points (basal and second insulin dose) vs 3 points (basal, low, and high dose) were not different provided the proper second dose was selected. The EC_{50} for insulin suppression of lipolysis correlated linearly with plasma triglycerides ($r = 0.52$, $P < .001$) and exponentially with insulin $sensitivity_{glucose}$ ($r = 0.70$, $P < .001$). We conclude that log transformation of insulin dose response data for FFA permits straightforward data analysis and simplifies the estimation of metabolically relevant parameters.

1. Introduction

Abnormal insulin regulation of free fatty acid (FFA) metabolism is increasingly recognized as an important problem in the context of obesity, insulin resistance, and type 2 diabetes mellitus. The euglycemic hyperinsulinemic clamp is often used as the experimental approach to quantify this process [1-8]. Advantages of the insulin clamp technique include the ability to measure individual, steady-state FFA rate of appearance (Ra) and good sensitivity as defined by the ability to distinguish between groups that are insulin-resistant vs insulin-sensitive with respect to regulation of lipolysis [1]. Investigators have used either a single insulin infusion dose [1,3] or multiple insulin doses [2,4-8] depending on the experimental question. The use of multiple doses is considered necessary to develop full insulin dose response characteristics for the suppression of lipolysis. In addition, because lipolysis is exquisitely sensitive to minor increases in plasma insulin concentration [4], very low initial insulin infusion rates are considered helpful in defining the dose-response relationships.

We [4] and others [2] have measured the insulin dose response characteristics of FFA kinetics by using multiple steps of an insulin clamp. With 4 to 5 data points it is possible to use power exponential formulas [4] or logistic regression [2] to assess each individual's insulin dose-response relationship. Unfortunately, these experimental protocols are complex, involving the infusion of somatostatin, growth hormone, and insulin [4] or requiring 2 separate study days [2]. In addition, the data analysis is difficult. Although these approaches have defined the exquisite sensitivity of lipolysis to insulin, the results could not be readily translated into an understanding of day-to-day regulation of FFA metabolism. A better understanding of implications of this information requires information regarding the insulinemic responses to daily activities (meals, physical exercise) as it relates to basal FFA availability. We

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considered that this question would not require the infusion of somatostatin to achieve very low insulin concentrations, especially if the derived index of FFA/insulin sensitivity data relates to associated metabolic indicators, such as triglyceridemia or insulin sensitivity, with respect to glucose metabolism.

Herein we present the results of studies examining the insulin regulation of FFA and glucose using 2 doses of insulin; an initial low infusion rate followed by a high-physiologic/low-pharmacologic insulin infusion rate chosen to maximally suppress lipolysis. The dose response characteristics of insulin vs glucose disposal were largely linear and the dose response characteristics for FFA were strikingly nonlinear. We used an analytical approach that apparently has not been previously applied to this problem: by log transforming both the x-axis (plasma insulin concentration) and the y-axis (palmitate concentrations or palmitate flux) the lipolysis dose-response relationships could be largely linearized. The exception appeared to be nonobese men, in whom the high dose of insulin appeared to be "too high." Additional studies were performed using a reduced second dose of insulin that improved the linearity of the log-transformed data.

2. Research design and methods

These studies were approved by the institutional review board at Mayo Clinic and informed written consent was obtained from all participants.

2.1. Subjects

Seventeen healthy, overweight, and obese volunteers (10 men, 7 women) with a BMI of 28.0 to 36.0 kg/m^2 and 20 healthy, nonobese (13 men, 7 women) age-matched volunteers participated in these studies. We initially sought to recruit according to the National Heart, Lung and Blood Institute Guideline definitions (normal weight, 18.5-24.9 kg/m^2; overweight, 25.0-29.9 kg/m^2; obese, $\geq$30.0 kg/m^2); however, 3 men who volunteered for our study had BMIs greater than 25.0 but had 22% or less body fat, indicating their excess weight was due to greater lean tissue, not excess fat tissue. Because their percent fat was more typical of what we find in normal-weight men, these volunteers were included in the "nonobese" group. All overweight/obese men (subsequently referred to as "obese") had greater than 28% body fat. All volunteers were weight stable for the previous 3 months and all had a normal blood cell count and biochemistry panel. Obese volunteers were recruited with the criteria that the waist-to-hip ratios (WHRs) must be greater than 0.95 for men or greater than 0.85 for women in order to select for more insulin-resistant individuals. Additional inclusion criteria were being nonsmokers, no regular medication use, and weight stable for the previous 3 months. All women were premenopausal and had a negative pregnancy test before the study. All of the volunteers for these studies were Caucasian.

2.2. Protocol

Total body fat and fat-free mass (FFM) were measured 1 week before the study by DXA (Lunar Radiation, Madison, WI). All participants were provided weight-maintaining meals (40% fat, 40% carbohydrate, 20% protein) at the Mayo Clinic General Clinic Research Center for 3 days before the study to assure consistent energy intake. All subjects maintained their usual level of physical activity and were asked not to participate in heavy exercise the last 3 days before the study. Each participant was admitted to the research center the evening before the insulin clamp study where they consumed a standard evening meal at 1800 hours and then consumed no additional food until the completion of the study the following day.

The morning after admission we performed a two-step hyperinsulinemic euglycemic clamp with palmitate and glucose tracers for measurement of basal and insulin-regulated palmitate and glucose kinetics. After a baseline blood sample was collected, an intravenous catheter was inserted in an antecubital vein for isotope infusions; blood samples were collected from an arterial catheter. Infusions of [3-^{3}H]glucose and [9,10-^{3}H]palmitate were started at 0630 and 0800 hours, respectively. After completion of all measurements, all catheters were removed and the participants were dismissed. Resting energy expenditure was measured during the basal time interval by using a DeltaTrac Metabolic Cart (Sensor Medics, Yorba Linda, CA). We now routinely measure resting energy expenditure to allow for sex-specific analysis of FFA flux data should it be necessary [9].

2.3. Glucose and palmitate turnover

All of the women, 7 of the nonobese, and 7 of the obese men underwent a 2-step euglycemic hyperinsulinemic clamp to assess basal and insulin-regulated glucose and palmitate kinetics. Regular insulin was infused at a rate of 0.25 mU/kg FFM per minute (step 1) and 2.5 mU/kg FFM per minute (step 2) and plasma glucose was "clamped" at approximately 5 mmol/L by infusion of 50% dextrose labeled with [3-^{3}H]glucose (74 μCi/100 g dextrose). Plasma glucose was measured in duplicate every 5 to 10 minutes (Beckman Instruments, Palo Alto, CA) to allow adjustment of the glucose infusion rate needed to maintain euglycemia. A priming dose of 12 μCi [3-^{3}H]glucose (New England Nuclear, Boston, MA) was given at 0630 hours and followed by continuous infusion (0.12 μCi/min) throughout the basal ($-$90 to 60 minutes) and the hyperinsulinemic periods (step 1, 60 to 180 minutes; step 2, 180 to 300 minutes) for determination of glucose turnover rates. Palmitate flux was measured by using a constant infusion of [9,10-^{3}H]palmitate (Amersham, Arlington Heights, IL) (0.3 μCi/min) at times 0 to 60, 120 to 180, and 240 to 260 minutes. This allowed 30 minutes for isotopic equilibration before blood sampling, which is all that was needed because of the brief (3-4 minutes) half-life of FFA.

Blood samples for measurements of palmitate concentration, specific activity, and glucose-specific activity were drawn before the first tracer infusion and at 10-minute intervals over the last 30 minutes of each infusion period.

We asked the male volunteers to participate in another set of studies using a reduced second dose of insulin. Two nonobese men and 5 obese men from the initial studies agreed to return for the second set of studies that was similar to the first, except that an infusion rate of 1.0 mU/kg FFM per minute was used as the second insulin dose. An additional 5 nonobese men and 2 obese men were recruited to have an equal number of men in the second set of studies. This allowed us to understand anomalies in the linearity of the log-transformed insulin/palmitate dose response characteristics from the 2.5 mU/kg FFM per minute dose of insulin. The men who participated in this second study also underwent measures of vascular regulation by angiotensin II [10].

2.4. Analysis of samples

Insulin concentrations were measured using chemiluminescent sandwich assays (Sanofi Diagnostics Pasteur, Chaska, MN). Plasma palmitate concentration and specific activity were determined by high-performance liquid chromatography using [$^2H_{31}$]palmitate as an internal standard [11], and plasma triglyceride concentrations were measured as previously described [11,12].

2.5. Statistics

Values are reported as means ± SEM. Comparisons between groups were performed using analysis of variance followed by Student *t* test or the Mann-Whitney 2-sample test if the analysis of variance indicated significant differences. Glucose disposal dose-response curves were evaluated by using linear regression analysis, whereas palmitate dose-response curves were evaluated by using-log transformed values. Correlations were evaluated by Pearson *r*, except if the relationship was clearly nonlinear and could not be linearized by using standard approaches, in which case we used exponential models using JMP 5.1 (SAS Institute, Cary, NC). *P* values less than .05 were considered statistically significant. The log-transformed insulin and palmitate values were used to calculate the slope and intercept for each individual's dose response; the insulin concentration resulting in 50% suppression of palmitate from basal values (EC_{50}) was calculated using this data. The insulin stimulation of glucose disposal for each participant was calculated as the slope of the glucose Rd (milligrams per kilogram FFM per minute) vs plasma insulin concentration; log transformation was not necessary for this data.

3. Results

3.1. Subject characteristics

The subject characteristics are provided in Table 1. The expected differences in body fat, body fat distribution, and resting energy expenditure were observed. The obese men were significantly hypertriglyceridemic compared with the other groups.

3.2. Plasma insulin concentrations

The individual plasma insulin concentrations and the group mean values are depicted in Figs. 1-4.

3.3. Glucose turnover results

Fig. 1A depicts the mean values for plasma insulin concentrations and glucose rates of disappearance (Rd) for the obese and nonobese men and women participating in the studies. The relationships were generally linear for each individual (data not shown) and for the average relationships for the different groups. Fig. 1B depicts the responses of the different groups to the 0.25 mU/kg FFM per minute dose of insulin. This panel shows that (1) the nonobese men and nonobese women respond almost identically to this dose, (2) the nonobese men and obese men respond similarly to this dose in both studies, and (3) the data points for obese women lay between those of the nonobese groups and the obese men.

Table 1
Subject characteristics

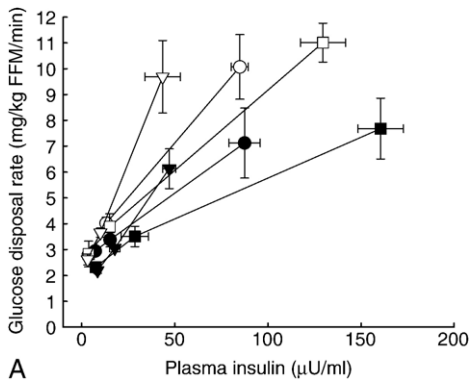
	Nonobese women (n = 7)	Obese women (n = 7)	Nonobese men (n = 13)	Obese men (n = 10)
Age (y)	27 ± 1	31 ± 3	30 ± 1	36 ± 3
BMI (kg/m^2)	22 ± 1	35 ± 1	24 ± 1	32 ± 1
Fat-free mass (kg)	42.3 ± 1.6	51.2 ± 1.6*	64.5 ± 3.7	69.0 ± 1.5*
% Body fat	31 ± 2	46 ± 1*	20 ± 1	31 ± 1*
Waist-hip ratio	0.78 ± 0.02	0.93 ± 0.02	0.86 ± 0.01	0.98 ± 0.02
Fasting plasma glucose	88 ± 2	92 ± 3	90 ± 1	92 ± 1
Fasting plasma triglyceride (mg/dL)	95 ± 15	122 ± 21	99 ± 17	252 ± 34†
Resting energy expenditure (kJ/d)	6456 ± 322‡	7372 ± 364	7519 ± 272	8368 ± 226†

Values are given as mean ± SEM. Statistical comparisons are not performed on variables that were used as selection criteria (BMI and waist-hip ratio). See the section Research design and methods for the statistical analysis approach.

* $P < .001$ vs nonobese.

† $P < .05$ vs all other groups.

‡ $P < .05$ vs obese men.



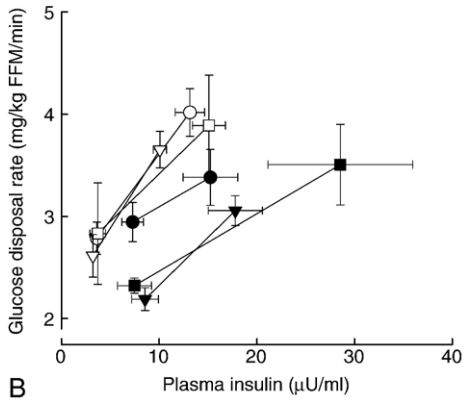
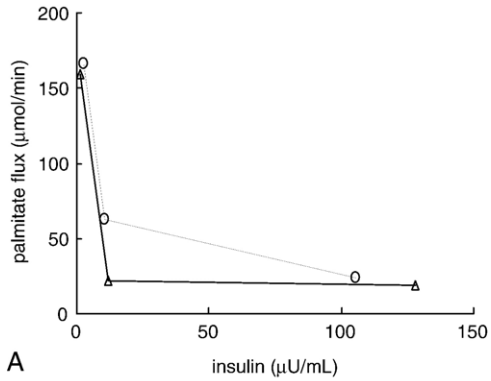


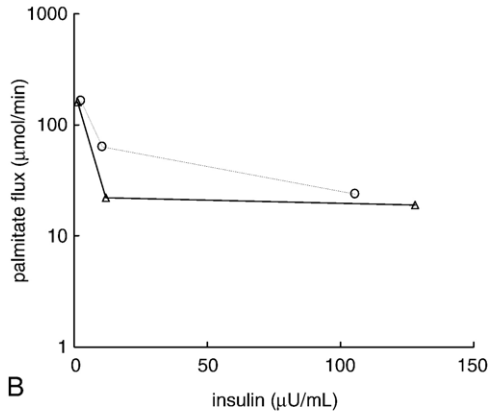
Fig. 1. A, Glucose disposal rates are plotted vs plasma insulin concentrations for nonobese men (open triangles and open squares), nonobese women (open circles), obese women (closed circles), and obese men (closed triangles and closed squares). The triangle symbols represent values from the study that used 1.0 mU/kg FFM per minute as the second, high dose of insulin. B, An expanded depiction of the response to the 0.25 mU/kg FFM per minute dose of insulin for the same groups using the same symbols.

3.4. Palmitate flux

Fig. 2 provides examples from 2 individual studies of nonobese men that display the relationship between plasma insulin concentrations and palmitate flux when neither plasma insulin nor palmitate flux (Fig. 2A) or palmitate flux only (upper right panel) were log transformed. Log transformation of both plasma insulin concentrations and palmitate flux frequently, but not always, linearized the relationship (Fig. 2B). The individual values for plasma insulin concentration and palmitate flux (both log transformed) for the studies using 2.5 mU/kg FFM per minute as the second insulin dose are provided in Fig. 3. These relationships appear generally linear using this means of data presentation for obese men (upper right panel) and for most of the obese women (lower right panel). For 2 of the 7 nonobese women (lower left panel) and 5 of the 7 nonobese men (upper left panel) most of the suppression of palmitate flux occurred at the low insulin dose. We suspected that the 2.5 mU/kg FFM per minute insulin dose created plasma insulin concentrations that were sufficiently far above the plateau of maximally suppressive insulin concentrations so that the result was a nonlinear insulin/palmitate flux relationship despite both axes being logarithmic.

Fig. 4 depicts the individual values for plasma insulin concentrations and palmitate flux on a log-log scale for the nonobese (upper left panel) and obese (upper right panel) men participating in the study that used a second insulin dose of 1.0 mU/kg FFM per minute. The palmitate flux/insulin concentration relationships are more predictably linear than those seen with the 2.5 mU/kg FFM per minute dose of insulin. The lower panel of Fig. 4 depicts the group mean relationships between palmitate flux and plasma insulin concentrations. The relationships for obese men





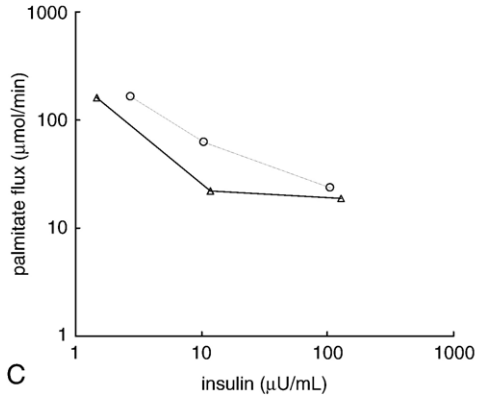


Fig. 2. Data points from 2 individual studies of nonobese men depicting the plasma insulin concentrations on the x-axis and palmitate flux on the y-axis. A, Both axes are plotted in a linear fashion. B, The insulin axis is linear and the palmitate axis is logarithmically transformed. C, Both axes are logarithmically transformed.

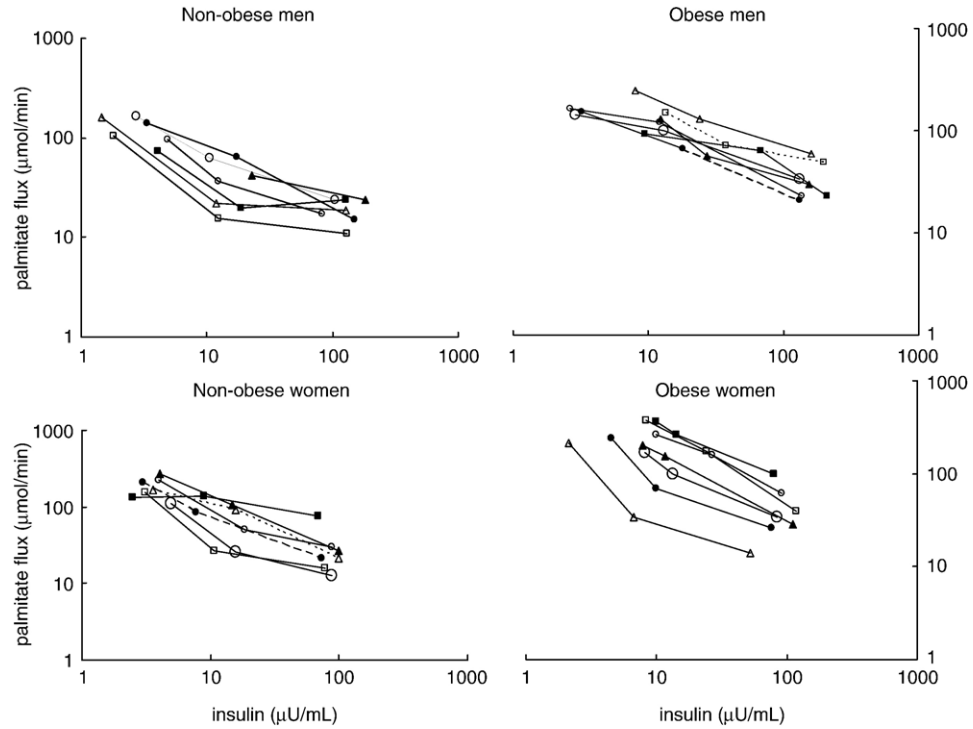


Fig. 3. Individual palmitate flux values (micromoles per minute) are plotted vs plasma insulin concentrations for the studies that used the 2.5 mU/kg FFM per minute second dose of insulin. Note that both palmitate flux and plasma insulin concentrations are plotted on logarithmic axes.

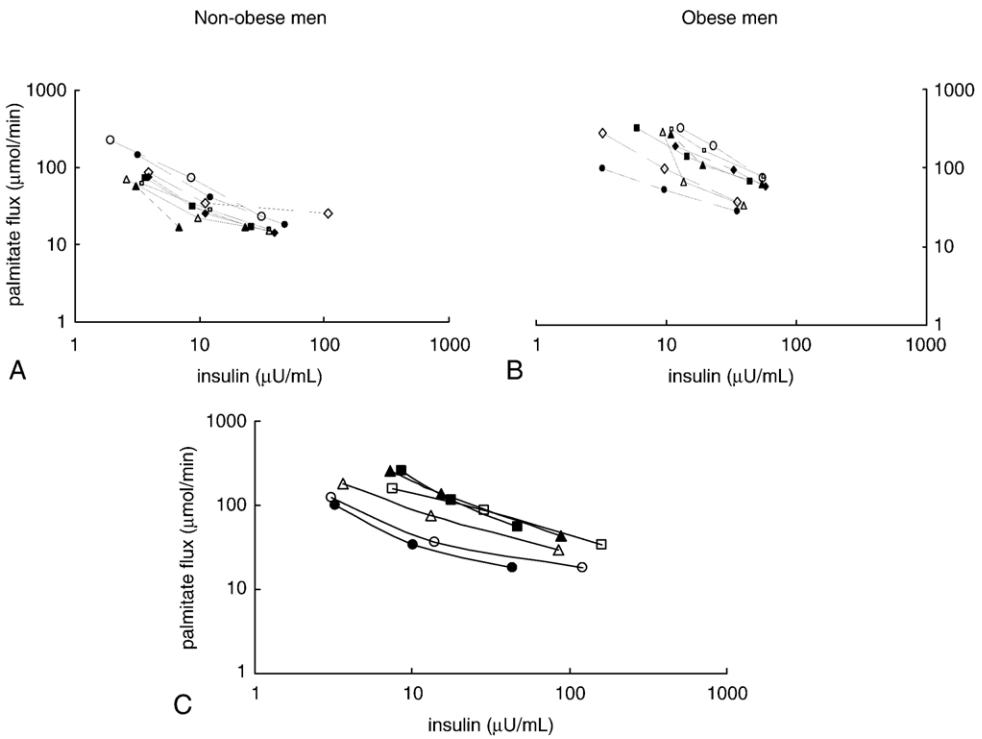


Fig. 4. Individual palmitate flux values (micromoles per minute) are plotted vs individual plasma insulin concentrations for the nonobese men (A) and obese men (B) participating in the study that used the 1.0 mU/kg FFM per minute second dose of insulin. Both palmitate flux and plasma insulin concentrations are plotted on log-transformed axes. C, Group mean plasma palmitate flux vs plasma insulin concentrations for the participants in all studies. The values for nonobese men are plotted in circles, for the obese men in squares, for the nonobese women in open triangles, and for the obese women in closed triangles. The closed circles and closed squares represent values from the studies that used 1.0 mU/kg FFM per minute as the second dose of insulin; the open symbols represent data from the studies that used 2.5 mU/kg FFM per minute as the second dose of insulin.

Table 2
Insulin regulation of palmitate flux slope and intercept parameters

	Slope (3-point)	Intercept (3-point)	Slope (2-point)	Intercept (2-point)
Insulin doses of 0.25 and 2.5 mU/kg FFM per minute				
Nonobese women	−0.68 ± 0.04	2.60 ± 0.180	−0.69 ± 0.049	2.66 ± 0.13
Obese women	−0.72 ± 0.09	2.94 ± 0.31†	−0.74 ± 0.09†	2.99 ± 0.24
Nonobese men	−0.50 ± 0.10*,†,‡	2.21 ± 0.23*,†,‡	−0.51 ± 0.10†,‡	2.30 ± 0.16†,‡
Obese men	−0.47 ± 0.07†,‡,§	2.54 ± 0.15‡	−0.48 ± 0.07‡,§	2.55 ± 0.19†,‡
Insulin doses of 0.25 and 1.0 mU/kg FFM per minute				
Nonobese men	−0.68 ± 0.10	2.23 ± 0.25	−0.69 ± 0.10	2.28 ± 0.22
Obese men	−0.86 ± 0.10§	3.12 ± 0.44§	−0.89 ± 0.29§	3.19 ± 0.50

Regression analysis was used to fit a line to the log insulin/log palmitate flux data for each participant by using either the basal, first-step, and second-step insulin clamp data (3-point approach) or just the basal and second-step insulin clamp data (2-point approach). The slopes and intercepts of the regression lines from the 3-point or 2-point approaches were compared by using paired *t* tests. Comparisons between nonobese and obese groups were done only if they received the same doses of insulin; nonpaired statistical approaches were used. Data are provided as the logarithmic values and are mean ± SD.

* $P < .05$ vs 2-point values.

† $P < .05$ vs nonobese women.

‡ $P < .05$ vs obese women.

§ $P < .05$ vs nonobese men.

and women are shifted up and to the right compared with nonobese women, who in turn are intermediate between the nonobese men and the obese groups. Plotting the values from the 2 study groups of nonobese men on the same graph emphasizes the plateau in suppression of lipolysis with insulin.

The apparent linearity of the log insulin/log palmitate plots prompted us to test whether the slope and intercept of the relationships were different if 2 points (basal and the highest insulin concentration) vs all 3 points were used to estimate these parameters. The results of this analysis are presented in Table 2. There were no significant differences between the slope and intercept calculated using 2 points and 3 points except for the nonobese men studied with a second insulin dose of 2.5 mU/kg FFM per minute; when studied with the 1.0 mU/kg FFM per minute insulin dose, the parameters were not different. The between-group differences in the slope and intercept of these relationships are also provided in Table 2. The same patterns of differences are present using either 2-point or 3-point approach.

The individual slope and intercept data from the 2.5 mU/kg FFM per minute insulin dose studies were used to calculate the insulin concentration that would suppress palmitate flux by 50% from basal values (EC_{50}). The EC_{50} values were not significantly different between nonobese men and nonobese women (9.0 ± 1.9 and 9.1 ± 1.1 μU/mL, respectively) or between obese men and women (33.3 ± 7.7 and 18.7 ± 3.8 μU/mL, respectively), but were greater in obese than nonobese groups (each <.05). The EC_{50} for the nonobese and obese men studied with the 1.0 mU/kg FFM per minute insulin dose were 7.6 ± 0.8 and 19.8 ± 2.9 μU/mL, respectively ($P < .01$, nonobese vs obese).

3.5. Plasma palmitate concentrations

The pattern of individual and group mean palmitate concentration responses to insulin largely mirrored the palmitate flux responses.

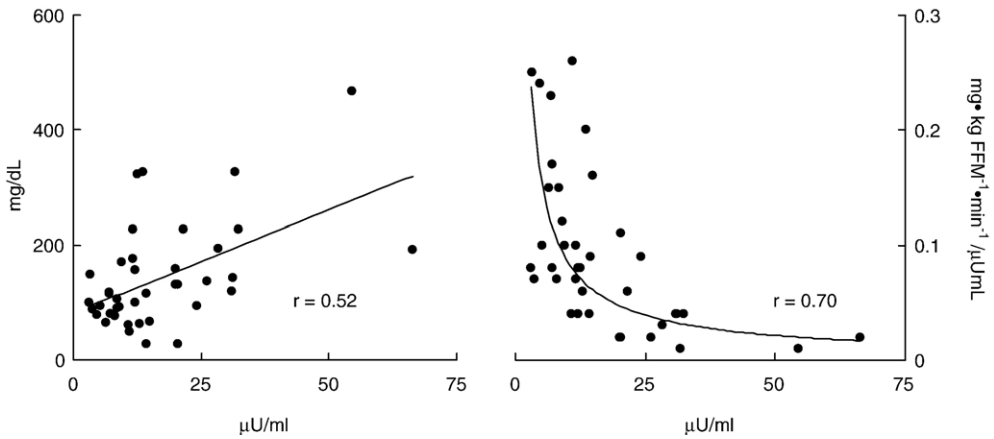


Fig. 5. The left panel displays the fasting plasma triglyceride concentrations plotted vs the EC_{50} insulin for suppression of lipolysis for all participants; the *r* value is the Pearson correlation coefficient using linear model assumptions. The right panel displays $Si_{glucose}$ vs the EC_{50} insulin for suppression of lipolysis for all participants; the *r* value is that obtained using an exponential regression model (see Results).

3.6. Relationship between insulin regulation of lipolysis indicators, triglycerides, and insulin sensitivity (glucose)

We examined whether the EC_{50} insulin values were related to other indices of metabolic health: fasting plasma triglyceride concentrations and insulin sensitivity$_{glucose}$ ($Si_{glucose}$). The latter parameter was taken as the increase in glucose Rd as a function of the increase in plasma insulin concentrations during the insulin clamp using linear regression analysis. The basal, first, and second insulin dose values were used to calculate $Si_{glucose}$.

Fasting plasma triglyceride concentrations collected on the day of the study were correlated ($r = 0.52$, $P < .001$, Fig. 5, left panel) with the EC_{50} insulin; the EC_{50} insulin from the 2-point dose response analysis was similarly correlated ($r = 0.54$, $P < .001$). $Si_{glucose}$ was correlated with the EC_{50} insulin ($r = 0.53$, $P = .0003$), although when using exponential models the correlation between $Si_{glucose}$ and EC_{50} insulin was much better ($r = 0.70$, Fig. 5, right panel). Comparable results were obtained when the 2-point method was used to calculate the EC_{50} insulin.

4. Discussion

Research measures of insulin regulation of lipolysis and glucose disposal are commonly used to describe the effects of obesity and diabetes in human and animal models. From previous studies, we anticipated that lipolysis would be very sensitive to insulin [2,4] and that glucose disappearance would be less sensitive. In these studies, glucose disposal increased in a largely linear fashion as insulin concentrations were increased up to 50 to 130 μU/mL; the data were relatively straightforward to analyze and display. In contrast, the lipolysis data were not linear and we did not have enough data points to analyze the response by using a power exponential or logistic regression model [2,4]. We found that log transforming both insulin concentration and palmitate data allowed us to analyze and present the lipolysis/insulin dose-response relationships by using simpler linear approaches. This logarithmic scaling approach is used to model several types of biological data [13] and worked well to linearize the dose-response relationships for the majority of individual data sets and for group mean data. The EC_{50} of insulin for suppression of palmitate flux could easily be calculated by using a 2-dose insulin clamp, and our data indicate that the EC_{50} of insulin can be accurately calculated from a 2-point data set (basal and 1 insulin dose). This further simplifies the experimental design for studies of lipolysis by avoiding the need for somatostatin infusion and avoiding the need to use multiple insulin doses.

A number of groups have studied the insulin dose response characteristics for suppression of lipolysis by using glycerol tracer methods [7,14], FFA tracer methods [2,4,5], or measures of FFA concentrations alone [8]. Typically, at least 3 doses of insulin are infused for variable periods to define the sensitivity of lipolysis to insulin, as estimated by the EC_{50}. The use of glycerol kinetics is confounded by the multiple sources of plasma glycerol, some of which may be highly suppressible by insulin (adipose tissue [15]); some are only moderately suppressible (intramyocellular [14]), whereas others are not insulin suppressible (VLDL-TG hydrolysis [16]). In addition, plasma glycerol is an imperfect marker of circulating fatty acid fuel availability [17], and abnormalities of FFA, not glycerol, availability are of primary interest in states of insulin resistance.

A commonly cited application of using logarithmic conversion of both axes is to understand the relationship of body size to resting metabolic rate [13], although a large number of cardiovascular and respiratory variables are better described using this approach [18]. Carpentier et al [8] estimated the slope of FFA suppression by insulin by plotting the change in insulin concentrations from 0 to 80 pmol/L (linear scale) vs the natural log of FFA concentrations. Stumvoll et al [14] examined the insulin dose response of glycerol kinetics by using log-transformed plasma insulin concentrations vs the percent suppression of glycerol flux. Although these approaches are similar to ours, we found that using the logarithm of the absolute insulin concentration and a wider range of insulinemia offers some advantages. These include the ability to easily calculate the EC_{50} for insulin suppression of lipolysis and, if a dose of insulin that achieves postprandial insulinemia is selected, an estimate of the suppression of lipolysis that will occur under day-to-day postprandial conditions.

The biological underpinnings that explain the differences between the insulin regulation of lipolysis vs glucose disposal are superficially apparent. Both in vitro [19] and in vivo [2,4,20] studies have demonstrated that very low concentrations of insulin can inhibit adipocyte lipolysis, whereas much higher concentrations are needed to stimulate muscle glucose uptake [21]. The extent of this difference is emphasized by the insulin effects on lipolysis being most easily described using logarithmic scaling, whereas glucose disposal relationships require no such data transformation. From a teleology perspective, the exquisite sensitivity of adipocyte lipolysis to the inhibitory effects of insulin is advantageous as regards protection from the adverse effects of excessive FFA release. Whereas low FFA concentrations secondary to moderate postprandial hyperinsulinemia are not harmful, a more graded response to insulin-mediated glucose disposal offers a lower likelihood of hypoglycemia.

In examining the doses of insulin that were used to suppress lipolysis in the present study, we noted that the 2.5 mU/kg FFM per minute infusion of insulin appeared to be substantially above the maximal suppressive dose for some individuals in each group and was routinely excessive for nonobese men. If we had performed a single-step insulin clamp using only this dose, we would have underestimated the insulin sensitivity for suppression of lipolysis in a number of participants. The slope and intercept parameters for insulin regulation of lipolysis were significantly different in the nonobese men studied with 2.5 mU/kg FFM per

minute of insulin when a 2-point approach was used compared with a 3-point approach (Table 2, top grouping), and the EC_{50} for insulin suppression of lipolysis was somewhat greater ($P = .08$) with the 2-point approach. On the other hand, the insulin infusion rate of 1.0 mU/kg FFM per minute achieved plasma insulin concentrations seen in the postprandial state and provided a good separation of groups with different insulin sensitivity with respect to lipolysis (Fig. 4, Table 2) and glucose disposal (Fig. 1). The 0.25 mU/kg FFM per minute insulin infusion rate did not appear to add significant definition to the insulin-lipolysis dose-response curves (Figs. 3 and 4).

The suppression of plasma FFA concentrations by insulin is thought to play an important role in allowing stimulation of glucose disposal [15] and regulating hepatic triglyceride production [22]. We found that the EC_{50} for insulin suppression of FFA flux (calculated using either the 2-point or the 3-point approach) was correlated both with fasting plasma triglyceride concentrations and with $Si_{glucose}$. Thus, we believe that this parameter of insulin regulation of lipolysis, which can be easily estimated by using the approach we describe, has physiological relevance. Of note, $Si_{glucose}$ was not significantly related to fasting plasma triglyceride concentrations (data not shown).

Although we suggest that the use of a single insulin infusion rate of 1.0 mU/kg FFM per minute will allow investigators to calculate important parameters related to the insulin regulation of lipolysis and to determine the degree of suppression of FFA likely to be encountered under typical postprandial circumstances, this approach has a drawback. This dose will not maximally suppress lipolysis in most nonobese and obese women or in obese men. If the goal of a study is to determine the maximum suppression of FFA flux it will be necessary to use a higher dose of insulin because logarithmic transformation of FFA and insulin will not allow maximum suppression to be calculated. Another limitation of this study is that we repeated the insulin clamp experiments using the 1.0 mU/kg FFM per minute dose only in men; we do not have data for comparison in women. From the linearity of the log-transformed dose-response relationships in women using the 0.25 and 2.5 mU/kg FFM per minute insulin doses, however, we did not believe we could justify performing the additional studies. Because the approach we describe seems to work well for insulin-sensitive nonobese and the insulin-resistant upper body obese, it should apply to populations who are in between in insulin sensitivity with respect to lipolysis. To the extent that more obese subjects, especially those with type 2 diabetes mellitus, are more insulin resistant with respect to suppression of lipolysis, we recommend testing this approach in such groups rather than assuming it is valid.

In summary, we report that logarithmic scaling techniques are helpful in analyzing insulin dose-response relationships for suppression of lipolysis, whereas straightforward linear approaches work well for glucose disposal data. These mathematical relationships likely reflect biologic differences in the regulation of these processes. We also suggest that a single insulin clamp infusion of 1.0 mU/kg FFM per minute will allow investigators to compare insulin regulation of both lipolysis and glucose metabolism in nonobese and obese individuals using robust pharmacological parameters. Although this dose may underestimate the insulin sensitivity of lipolysis in some nonobese/insulin-sensitive individuals, the EC_{50} values obtained still are highly correlated with triglyceridemia and glucose disposal. This approach also provides good definition of group mean data and achieves insulin concentrations and FFA concentrations seen after meal ingestion [23], making it a relevant dose with respect to daily human physiology. This information should allow experiments examining the insulin regulation of lipolysis to be conducted in a simpler fashion without the loss of important data.

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