Five Weeks of Insulin-Like Growth Factor-I Treatment Does Not Alter Glucose Kinetics or Insulin Sensitivity During a Hyperglycemic Clamp in Older Women

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Insulin sensitivity and the activity of the hypothalamic-growth hormone (GH)- insulin-like growth factor-I (IGF-I) axis both decline with age. Treatment with IGF-I increases insulin sensitivity in healthy young subjects. We hypothesized that increasing plasma IGF-I in postmenopausal women to levels characteristic of young women would enhance insulin sensitivity. To test the hypothesis, fasting glucose kinetics and insulin sensitivity were measured in 24 healthy, normoglycemic, postmenopausal women before and after 5 weeks of treatment with either recombinant human (rh)IGF-I (15 μg/kg body weight/d twice daily) or placebo in a double-blind study. Diet energy content and composition were rigidly controlled to maintain energy balance. A hyperglycemic clamp (8 mmol/L) coupled with stable isotope infusion ([6,6^{2}H]glucose) was performed before and after treatment to assess whole-body insulin sensitivity; defined as the glucose rate of disappearance (Rd) or rate of infusion (GRIF) scaled to the steady-state insulin concentration (I). There were no differences in fasting glucose or insulin concentrations, glucose kinetics, or glucose oxidation after either treatment. During the clamps, steady-state insulin concentrations with placebo (pre = 151 ± 28 pmol/L, post = 173 ± 31 pmol/L) were slightly different than with IGF-I (pre = 182 ± 37 pmol/L, post = 163 ± 33 pmol/L), but the variations were not significant. No significant changes in whole-body insulin sensitivity were observed after treatment with IGF-I, calculated as Rd/I (pre = 17.7 ± 2.6 μg/kg/min/pmol/L, post = 19.3 ± 2.0 μg/kg/min/pmol/L for IGF-I *v* pre = 24.2 ± 2.5 μg/kg/min/pmol/L, post = 22.8 ± 3.4 μg/kg/min/pmol/L for placebo) or as GRIF/I (pre = 18.0 ± 3.9 μg/kg/min/pmol/L, post = 22.3 ± 3.5 μg/kg/min/pmol/L for IGF-I *v* pre = 26.4 ± 6.2 μg/kg/min/pmol/L, post = 26.9 ± 4.8 μg/kg/min/pmol/L for placebo). Baseline insulin sensitivity in women using hormone replacement therapy (HRT, n = 15) was similar to nonusers (n = 9), but HRT users derived a greater portion of energy expenditure from carbohydrate oxidation compared with nonusers. HRT use had no impact on the response to IGF-I. Overall, we observed subtle, but physiologically insignificant, variations after IGF-I treatment in the direction of enhanced insulin sensitivity. The data suggest that 5 weeks of low-dose rhIGF-I treatment has no material influence on whole-body insulin sensitivity in normoglycemic postmenopausal women.

INSULIN-LIKE growth factor-I (IGF-I) stimulates glucose uptake from the blood,[1-6] enhances glucose utilization by peripheral tissues,[1-5] and suppresses hepatic glucose production in a manner similar to that of insulin.[2-4] Low circulating levels of IGF-I are associated with a greater risk for glucose intolerance,[7] and IGF-I function is abnormal in insulin-resistant states, such as type-2 diabetes mellitus (T2DM).[8] In nondiabetic subjects, IGF-I administration increases peripheral insulin sensitivity (defined as the rate of blood glucose uptake at any given plasma insulin concentration).[3-6] In subjects with T2DM, the same effect has been reported,[2,9,10] although individuals with peripheral insulin resistance also appear to have subnormal IGF-I–stimulated glucose uptake.[1,4]

The age-related reduced activity of the hypothalamic growth hormone (GH)-IGF-I axis known as the "somatopause" results in lower circulating levels of IGF-I in older (>60 years), as compared with younger (<40 years) adults.[11,12] Aging also appears to erode tissue sensitivity to the actions of insulin,[13,14] at least in those individuals who do not maintain high levels of physical activity.[13,15,16] If low circulating levels of IGF-I are an important contributor to the declining insulin sensitivity that occurs with aging, then supplementing elderly individuals with exogenous IGF-I sufficient to restore "young" serum concentrations should enhance insulin sensitivity. Because high doses of subcutaneous IGF-I (240 to 320 μg/kg/d) given over several weeks to individuals with T2DM caused adverse effects (mainly hypoglycemia) that required early termination of a large clinical trial,[10] a much lower dose (30 μg/kg/d) was chosen for the present study. Previously, we showed that 30 μg IGF-I/kg/d was sufficient to increase protein synthesis[17] and cause accretion of lean tissue[18] in a group of older women. The primary aim of the present study was to directly and quantitatively assess the influence of short-term treatment with low-dose recombinant human (rh)IGF-I on insulin sensitivity during a reproducible glucose challenge (hyperglycemic clamp) in older women while rigidly controlling for potentially confounding variations in dietary energy and composition.

Postmenopausal women have lower insulin sensitivity than younger premenopausal women, but the difference appears to be attributable to age rather than to menopausal status per se.[14,19] In animal studies, insulin action is enhanced by estrogen and attenuated by progesterone,[20] but the results from studies of hormone replacement therapy (HRT) in postmenopausal women are ambiguous. Although some data suggest insulin sensitivity is higher with HRT,[21] other investigators have reported no difference[22] or decreased insulin sensitivity with HRT.[23,24] Given the potential for interactions between exogenously administered IGF-I and estrogen or estrogen/proges-

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Submitted December 19, 2002; accepted March 10, 2003.

Supported by Grant No. AG-10999 from the National Institutes of Health (NIH) and the Medical Research Service of the Department of Veterans Affairs.

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0026-0495/03/5209-0044$30.00/0

doi:10.1016/S0026-0495(03)00166-5

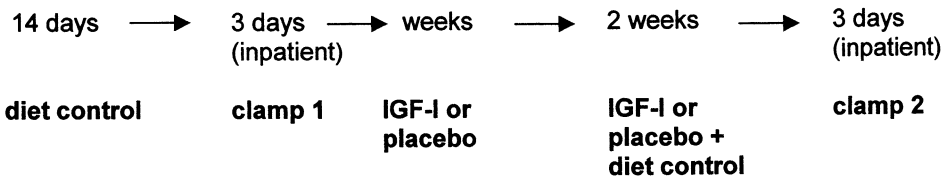

Fig 1. Schematic overview of the study design.

terone, an exploratory aim of this study was to compare the insulin sensitivity response to IGF-I in women using HRT with those who were not treated.

MATERIALS AND METHODS

Subjects

Thirty healthy, postmenopausal women over 60 years of age were recruited from the Palo Alto area. All were nonsmoking and free of chronic or systemic disease, including diabetes, coronary heart disease, and uncontrolled hypertension or hyperlipidemia. Women who were using HRT were admitted into the study as long as they had been on a stable dose of HRT for at least 1 year. In 6 women (4 in the placebo group, 2 in the IGF-I group), catheterization problems or other procedural difficulties precluded obtaining complete data sets for all relevant preintervention and postintervention tests, and the final sample size was 24 (11 placebo, 13 IGF-I). In the placebo group 8 of 11 subjects were HRT users and 7 of 13 subjects in the IGF-I group used HRT. Approximately equal numbers used estrogen alone, daily estrogen and progestin, or cyclic estrogen and progestin; with no obvious bias in the distribution between the IGF-I and placebo groups.

The protocol was approved by the administrative Panel for the Protection of Human Subjects in Research at Stanford University, and each volunteer gave written informed consent. At an initial visit, potential subjects completed a medical history questionnaire, had a physical examination, including a resting and exercise electrocardiogram (ECG), a standard fasting laboratory blood panel and urinalysis was performed, and 2-hour postprandial blood glucose was measured. Subjects were also required to have a normal mammogram and Pap smear before study enrollment.

Overview of Study Design and Drug Intervention

Participants were tested at baseline and after 5 weeks of treatment with IGF-I or placebo. For 2 weeks before and during the testing period, subjects consumed a standardized diet (described in more detail below). Three to 4 days before each test, subjects were admitted as in-patients to the Aging Studies Unit (ASU). A schematic overview of the study design is shown in Fig 1.

Subjects were instructed by the nursing staff on how to give themselves the appropriate dose of IGF-I or placebo by subcutaneous injection, and they began the injections immediately after the baseline testing was completed. Subjects remained in the ASU for the first 3 injections (approximately 24 hours) to be observed in case of adverse events. During that time, blood glucose was monitored with a One Touch II glucometer (LifeScan, Milpitas, CA), and juice was available to treat hypoglycemia.

Recombinant human (rh)IGF-I and placebo were donated by Genentech (South San Francisco, CA) and administered under IND # 36944. IGF-I (15 μg/kg body weight) or placebo was injected twice daily and the study was double blinded. The dose of IGF-I was selected because it was previously shown to be well tolerated and to alter body composition[18] and protein turnover[17] in postmenopausal women. Most of the women tested in this study were also involved in a parallel investigation centered on the effects of long-term IGF-I supplementation on body composition, bone density, and psychologic measures, and details regarding ensuring compliance, monitoring adverse events, etc are described elsewhere.[25]

Dietary Control

Subjects consumed a standardized diet intended to maintain body weight and control nutrient intake for 2 weeks before the pre- and posttreatment measures. Basal energy expenditure was estimated using the Harris-Benedict equation, and caloric need was calculated by multiplying the basal energy expenditure (BEE) by an activity factor of 1.5 to 1.6.[25] Subjects were weighed every 2 days during the 7-day stabilization period, and energy intake was adjusted as needed. The diet was composed of 1.0 to 1.2 g/kg body weight protein, 30% of energy as fat and carbohydrate contributing the balance of calories (approximately 55%). A daily calcium and mineral supplement was provided by Shaklee (San Francisco, CA). The diet provided 100% of the recommended dietary allowances for nutrients. Subjects ate the same foods every day during the 2-week diet control periods.

Procedures

Subjects fasted after 10 PM the day before testing. The next morning, a catheter was inserted into a forearm vein for blood sampling. A second catheter was placed in the antecubital vein of the contralateral arm for infusion of stable isotope. The hand and wrist were kept warm with a heating pad to stimulate arteriovenous mixing. After a blood sample was collected for determination of background isotopic enrichment, a priming bolus of 200 mg [6,6^{2}H]glucose in 0.9% sterile saline was rapidly infused into the venous catheter. After the bolus, a continuous infusion of [6,6^{2}H]glucose was started at a rate of 1.7 mg/min with a peristaltic infusion pump. Expired breath was collected between minutes 75 and 85 for analysis of O_2 and CO_2 concentration by indirect calorimetry (Ametek, Paoli, PA). Blood samples were collected at 70, 80, and 90 minutes after the start of the infusion.

Immediately after the 90-minute blood sample, the infusion rate of 6,6^{2}H-glucose was increased to 3.0 mg/min to account for the increase in glucose turnover in the insulin-stimulated condition. At the same time, an infusion of 20% dextrose in sterile saline was started at an average rate of 200 mg glucose/kg body weight for 10 minutes, with the goal of increasing plasma glucose concentration to approximately 8.0 mmol/L (150 mg/dL). After 10 minutes of infusion, small blood samples (0.5 mL) were taken every 5 minutes and glucose concentrations were rapidly analyzed. Adjustments to the glucose infusion rate were made to achieve and maintain a blood glucose concentration close to 8.0 mmol/L using negative-feedback algorithms adapted from those originally formulated by DeFronzo et al.[26] Using this method, plasma glucose concentrations reached a steady state after approximately 30 to 60 minutes. Blood samples were taken at 15, 30, 45, 60, 75, 90, 100, 110, and 120 minutes, and expired breath was collected from minutes 95 to 105.

Biochemical Assays

Collection tubes for glucose analysis contained sodium fluoride to inhibit glycolysis, and the protease inhibitor, aprotinin, was added to collection tubes to preserve insulin structure. Blood samples were kept ice-cold after collection and plasma separated by centrifugation as soon

Table 1. Subject Characteristics and Blood IGF-I Profiles Before and After Treatment With IGF-I or Placebo

	IGF-I (n = 13)		Placebo (n = 11)	
	Baseline	1 Month	Baseline	1 Month
Age (yr)	69.0 ± 2.4		68.1 ± 2.1	
Height (cm)	162.2 ± 1.5		164.2 ± 1.0	
Weight (kg)	66.2 ± 3.5	66.4 ± 3.7	66.3 ± 2.6	65.8 ± 2.6
BMI (kg/m^2)	25.2 ± 1.4	25.2 ± 1.5	24.6 ± 1.0	24.5 ± 1.0
Body fat (%)	33.2 ± 2.6	32.5 ± 2.7	33.1 ± 1.8	32.6 ± 1.8
IGF-I (ng/mL)	83.6 ± 15.0	233.1 ± 30.8*	74.3 ± 13.5	96.1 ± 15.7
IGFBP-3 (ng/mL)	2,419 ± 275	2,350 ± 239	2,148 ± 252	2,138 ± 171
IGFBP-1 (ng/mL)	85.6 ± 9.2	95.2 ± 8.9	99.3 ± 12.7	99.0 ± 11.5

*Significantly different from the baseline condition.

as feasible. Plasma was kept at −80°C until analyses were performed. Plasma concentrations of glucose were measured by the glucose oxidase method using a Beckman II glucose analyzer (Fullerton, CA). Antibody-specific insulin concentrations were measured by radioimmunoassay (DSL, Webster, TX). IGF-I, insulin-like growth factor-binding protein (IGFBP-1) and IGFBP-3 were assayed as previously described.[27] To prepare samples for the measure of glucose isotopic enrichment, plasma was deproteinized, freeze-dried, dissolved in acetic anhydride/pyridine (2:1), dried under a stream of nitrogen, and reconstituted in 100 μL ethyl acetate. The samples were injected and penta-acetate derivatives separated using a Model 5890 gas chromatograph with spectra recorded on a Model 5989A mass spectrometer (both Hewlett-Packard Analytical, Wilmington, DE). Selected ion monitoring was used to compare the abundance of the unlabeled fragment (m/z = 331) with that of the di-deuterated isotopomer (m/z = 333). After correcting for background enrichment, the abundance of 6,6^{2}H-glucose was expressed relative to total glucose species.

Calculations and Methodologic Considerations

The rate at which glucose was taken up from the blood (rate of disappearance [Rd]) and replaced by the liver (rate of appearance [Ra]) was calculated using nonsteady-state equations designed for use with stable isotopes:

$$\text{blood glucose Ra (mg/min)} = \frac{F - V[(C_1 + C_2)/2][(IE_2 - IE_1)/(t_2 - t_1)]}{[(IE_2 + IE_1)/2]} \quad \text{(a)}$$

$$\text{blood glucose Rd (mg/min)} = Ra - V[(C_2 - C_1)/(t_2 - t_1)]. \quad \text{(b)}$$

F represents the isotopic infusion rate, IE_1 and IE_2 are the isotopic enrichments of plasma [6,6^{2}H]glucose at timepoints t_1 and t_2, respectively, C_1 and C_2 are the concentrations of plasma glucose at t_1 and t_2, and V is the estimated volume of distribution for glucose of 100 mL/kg.

The plasma insulin concentrations (I), glucose rates of infusion (GRIF), and glucose Rd measured during minutes 90 to 120 of the clamp were averaged and used to calculate insulin sensitivity. Whole-body insulin sensitivity was defined as the rate of glucose uptake per unit insulin. Two partly independent measures of whole-body insulin sensitivity were obtained: (1) mean glucose rate of infusion/mean plasma insulin concentration (GRIF/I) and (2) glucose rate of disappearance/mean plasma insulin concentration (Rd/I).

During the clamp, incomplete suppression of hepatic glucose production (HGP) was termed residual HGP and calculated from: glucose Rd − GRIF. Residual HGP is an indicator of hepatic insulin resistance and is presented relative to the preclamp value as (residual HGP_{clamp}/HGP_{basal}) · 100.

The rates of carbohydrate and lipid oxidation during minutes 90 to 120 of the clamp were calculated from gas exchange data as: glucose oxidation (g/min) = [(VCO_2/VO_2 − .707)/.29] · VO_2 · 5.05 kcal/L · 4 kcal/g; lipid oxidation (g/min) = {1 − (VCO_2/VO_2 − .707)/.29]} · VO2 · 4.68 kcal/L · 9 kcal/g.

Statistical Analysis

Data were analyzed using Statistical Analysis Software (SAS Institute, Cary, NC). Differences between treatment groups and over time for all variables were tested by analysis of variance with repeated measures using the mixed model (PROC-mixed) procedure of SAS. Tukey post hoc tests were used to determine where significant differences occurred. The α level was set at $P < .05$. Pearson product-moment analyses were used to assess correlation between changes in plasma IGF-I and selected criterion measures related to glucose and insulin metabolism before and during the hyperglycemic clamp. All data are reported as mean ± SEM.

RESULTS

Body Weight and Composition

As expected based on the design of the study, body weight and composition did not change between the pre- and posttreatment tests in either the placebo or the IGF-I group (Table 1).

Plasma IGF-I Concentration

As shown in Table 1, the plasma concentration of IGF-I did not change in the placebo group, but increased by almost 3-fold in the IGF-I–supplemented group. Plasma concentrations of IGFBP-3 and IGFBP-1 were unchanged over time in both groups.

Fasting Glucose and Insulin

Fasting concentrations of plasma glucose and insulin before and after the treatments are shown in Table 2. All individuals had normal fasting glucose concentrations (3.9 mmol/L < [glucose] < 5.9 mmol/L) before treatment. One subject in the placebo group had fasting hyperinsulinemia pre- (133 pmol/L = 22 μU/mL) and posttreatment (128 pmol/L = 21 μU/mL). Treatment with either placebo or IGF-I had no effect on fasted glucose or insulin concentrations.

Fasting Glucose Kinetics

Glucose Ra and Rd were virtually identical, and only the Ra data are shown in Table 2. Glucose Ra (fasting hepatic glucose production) was unchanged by treatment with either placebo or IGF-I. Respiratory exchange ratios (Table 2) were also very similar pre- and posttreatment in both groups.

Table 2. Summary of Selected Outcome Variables Before and After Treatment With IGF-I or Placebo

	IGF-I (n = 13)		Placebo (n = 11)	
	Baseline	1 Month	Baseline	1 Month
Fasting glucose (mmol/L)	4.96 ± 0.12	4.96 ± 0.11	4.99 ± 0.16	4.81 ± 0.16
Fasting insulin (pmol/L)	41 ± 5	37 ± 5	47 ± 11	46 ± 11
Fasting glucose Ra (mg/kg/min)	1.92 ± 0.11	1.88 ± 0.08	2.06 ± 0.10	2.04 ± 0.09
Fasting RER	0.85 ± 0.03	0.84 ± 0.03	0.83 ± 0.02	0.84 ± 0.02
Clamp RER	0.91 ± 0.02	0.91 ± 0.03	0.90 ± 0.02	0.89 ± 0.02
Clamp HGP (mg/kg/min)	0.38 ± 0.24	0.33 ± 0.16	0.47 ± 0.28	0.09 ± 0.08
% HGP suppression	91 ± 6	91 ± 4	87 ± 7	96 ± 6

Glucose and insulin responses to the clamp: As shown in Fig 2A and C, plasma glucose values reached a steady-state value after approximately 45 minutes in both the placebo and IGF-I groups and remained constant for the remainder of the clamp. There were subtle differences in the steady-state plasma glucose levels achieved, but, overall, glucose values were very similar in all conditions. Plasma insulin concentrations plateaued after 75 to 90 minutes in each condition (Fig 2B and D). The mean steady-state insulin concentration appeared to be slightly higher after placebo, but marginally lower after IGF-I. There were no significant differences among conditions however, which may be partially explained by the considerable interindividual variability in the insulin response to the glucose infusion.

Clamp Glucose Kinetics

As expected, the steady-state condition achieved during the clamp caused glucose Ra and Rd to be essentially identical

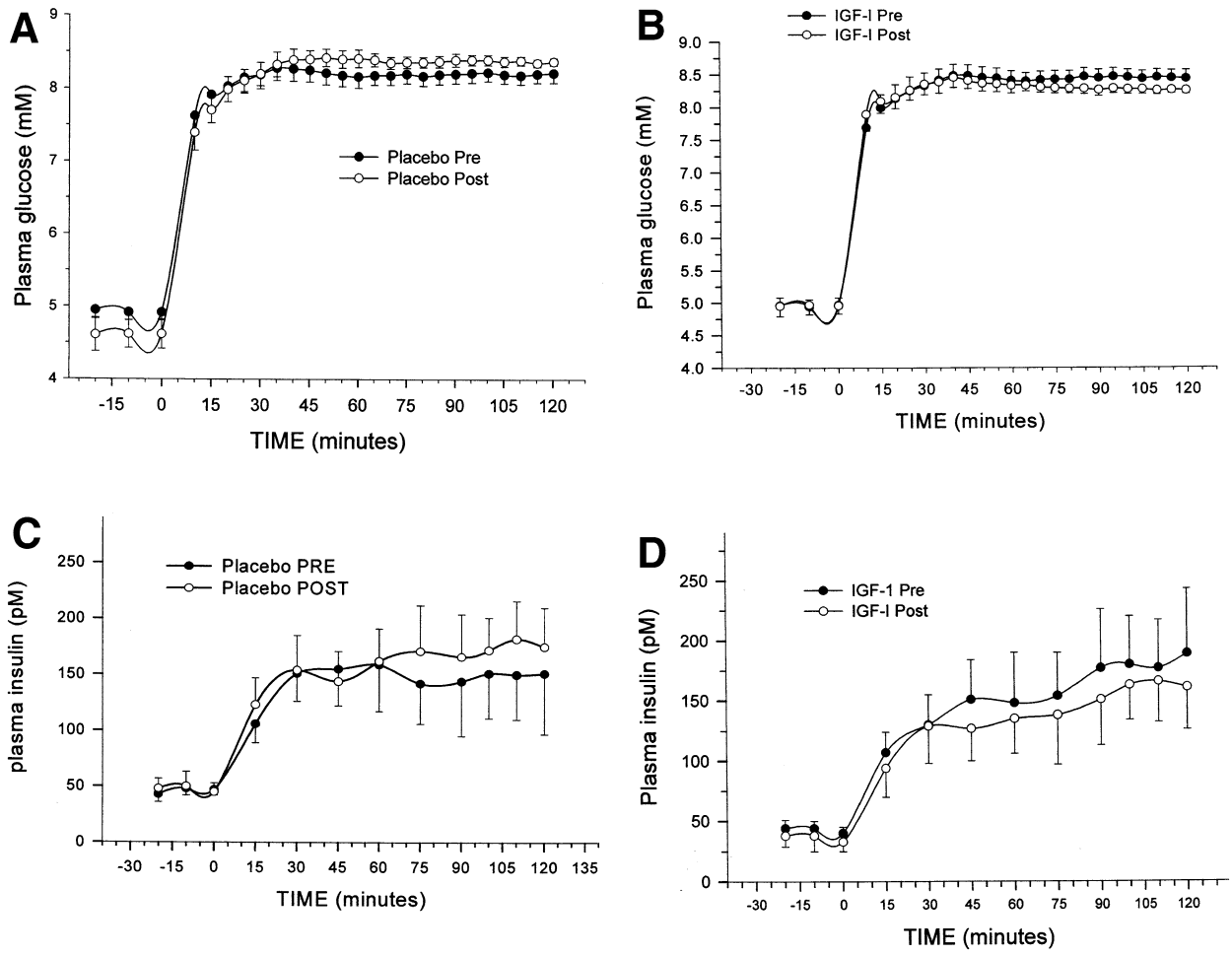

Fig 2. (A) Plasma glucose and (C) insulin concentrations (B and D) during the 120-minute hyperglycemic clamp before (PRE) and after (POST) treatment with either placebo or IGF-I.

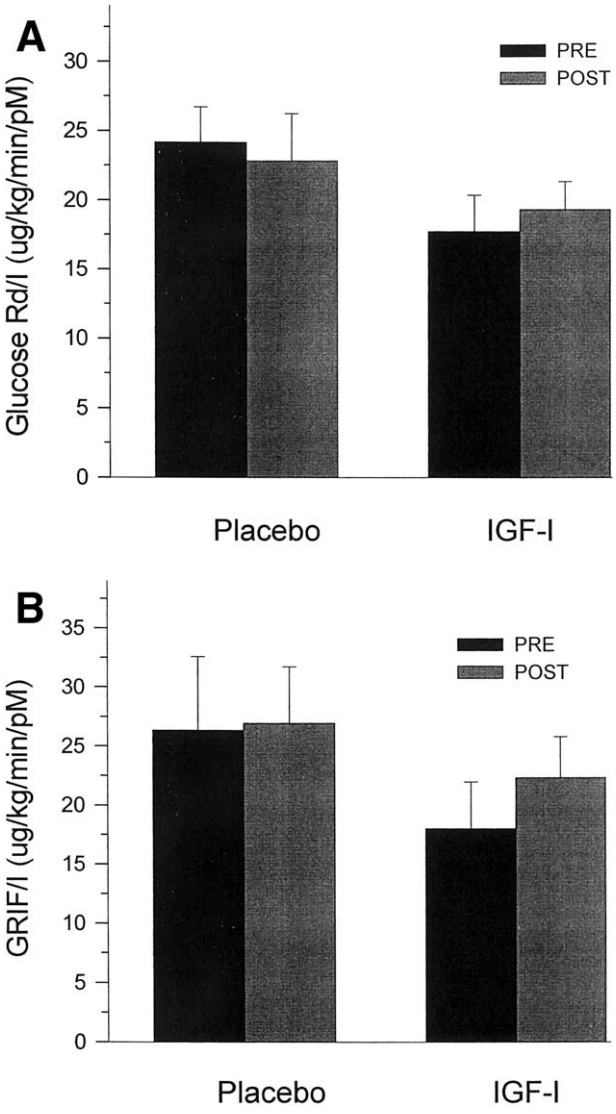

Fig 3. Whole-body insulin sensitivity defined as (A) the glucose rate of disappearance per unit plasma insulin concentration (Rd/I) and (B) the GRIF per unit plasma insulin concentration (GRIF/I) before (PRE) and after (POST) treatment with either placebo or IGF-I.

(differed by $< 1\%$). Glucose Rd did not change in either the placebo (pre = 3.65 ± .24 mg/kg/min, post = 3.95 mg/kg/min) or IGF-I condition (pre = 3.23 ± 22 mg/kg/min, post = 3.15 mg/kg/min). Similarly, the GRIF was not different after either placebo (pre = 3.98 ± .58 mg/kg/min, post = 4.66 ± .49 mg/kg/min) or IGF-I (pre = 3.28 ± .32 mg/kg/min, post = 3.64 ± .37 mg/kg/min) treatment.

Insulin Sensitivity

When scaled to the steady-state plasma insulin concentration, there were no differences in glucose uptake measured either by isotope dilution (Rd/I, Fig 3A) or by GRIF/I rate (Fig 3B). Results generated by the 2 independent methods of estimating whole-body insulin sensitivity were consistent, strongly suggesting that insulin sensitivity was not influenced by either treatment. We clamped blood glucose concentrations at 8 mmol/L with the expectation that this modest level of hyperglycemia would only partially suppress hepatic glucose production and allow us to determine the effects of treatment on hepatic insulin sensitivity. Unfortunately, HGP was suppressed by $\geq 90\%$ in each condition (Table 2), and so it is difficult to draw conclusions, but there does not appear to be any difference between treatments

Substrate Oxidation

There were no differences in the respiratory exchange ratio (RER) due to treatment in either group (data not shown). The ratio of carbohydrate to fat oxidation (Fig 4) was almost identical pre- and posttreatment in both the placebo and IGF-I condition.

Effects of HRT

A comparison between subjects using HRT (n = 15) versus those not using HRT (n = 11) on selected baseline variables related to insulin sensitivity is shown in Table 3. There were no differences between the 2 subgroups. HRT users derived a greater percentage of total energy from carbohydrate oxidation compared with nonusers (n = 9) at baseline. After IGF-I treatment, any initial differences between group means in HRT users versus nonusers were still apparent, with no obvious pattern toward accentuation or diminution (data not shown).

Correlations Between Plasma IGF-I and Glucose Metabolism

Correlation analyses were performed to assess whether there was any dose-response relationship between increases in

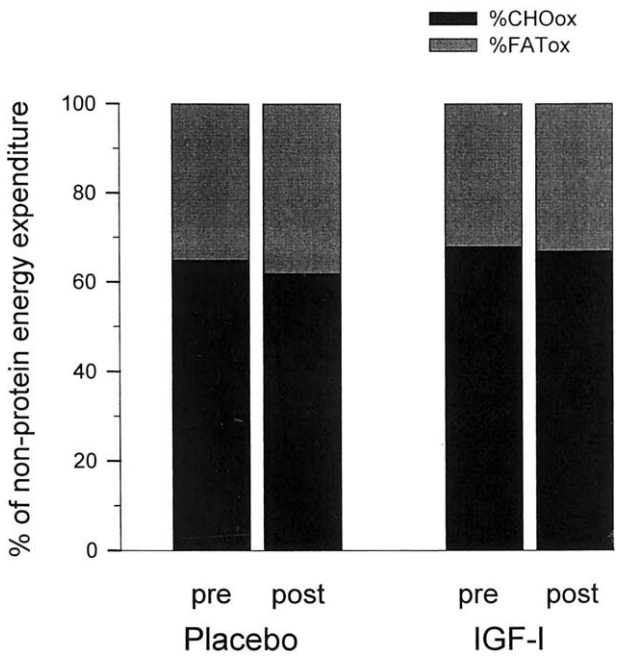

Fig 4. Contribution of carbohydrate and lipid oxidation (as % of total nonprotein energy expenditure) during the hyperglycemic clamp before (PRE) and after (POST) treatment with either placebo or IGF-I.

Table 3. Comparison of Major Outcome Variables Between Subjects Using HRT (n = 15) and Nonusers (n = 9) at Baseline

Measure	HRT User	Non user	HRT *v* Non user
Fasting glucose (mmol/L)	4.84 ± 0.12	5.08 ± 0.11	$P = .045$
Fasting insulin (pmol/L)	41 ± 4	41 ± 8	NS
HGP (mg/kg/min)	2.02 ± 0.10	1.89 ± 0.12	NS
Fasting CHO oxidation (% of total EE)	56 ± 6	35 ± 7	$P = .009$
Clamp glucose (mmol/L)	8.31 ± 0.11	8.23 ± 0.08	NS
Clamp insulin (pmol/L)	160 ± 29	172 ± 63	NS
Clamp Rd/I (μg/kg/min/pmol/L)	37.4 ± 8.7	42.9 ± 13.8	NS
Clamp GRIF/I (μg/kg/min/pmol/L)	38.2 ± 9.4	34.3 ± 9.5	NS
Residual HGP (% of fasting)	5.0 ± 3.7	10.4 ± 8.4	NS
Clamp CHO oxidation (% of total EE)	72.2 ± 6.1	59.0 ± 6.9	$P = .046$

Abbreviations: CHO, carbohydrate; NS, not significant; EE, energy expenditure.

plasma IGF-I concentration and changes in glucose kinetics or insulin sensitivity. There was no significant correlation between the change in plasma IGF-I levels and the magnitude of the change in fasting plasma insulin, fasting hepatic glucose production, clamp GRIF/I, or Rd/I. All correlation coefficients were low, with $r < .35$.

DISCUSSION

The main findings from this study were that 1 month of low-dose IGF-I supplementation did not significantly alter fasting glucose kinetics or insulin sensitivity in older women. The lack of response was observed in both users and nonusers of estrogen replacement therapy (ERT). These results do not support the hypothesis that IGF-I supplementation would increase insulin sensitivity in healthy, non-obese older women. The discordance between the results from the present study and prior investigations is likely attributable to differences in IGF-I dose, duration of treatment, and blood IGF-I levels achieved.[28,29]

IGF-I and Insulin Sensitivity

The experimental design of the current study was crafted to increase plasma IGF-I concentrations in older women to levels characteristic of young women. The intent was to "restore" IGF-I levels to physiologic, not pharmacologic, concentrations while minimizing the common side effects (eg, hypoglycemia, edema) of high-dose IGF-I treatment. Posttreatment IGF-I concentrations averaged 233 ± 31 ng/mL, which is close to the concentration (≈250 ng/mL) characteristic of young adults. Side effects (reported fully in Friedlander et al[25]) were mild. In contrast, when a positive impact of IGF-I on insulin sensitivity has been observed, IGF-I was administered by continuous infusion[3-6] or injecting doses >150 μg/kg/d[2,9,10] of IGF-I. The increase in IGF-I concentrations from baseline was greater, and posttreatment IGF-I levels were considerably higher in the prior studies. In nondiabetic subjects, baseline IGF-I concentrations between 100 to 300 ng/mL were boosted more than 4-fold to at least 440 ng/mL and as high as 1,200 ng/mL.[3-6] In subjects with T2DM, initial IGF-I concentrations tended to be low (60 to 150 ng/mL) and were increased by about 3-fold in response to high-dose subcutaneous injections.[2,9,10] Taken together, these data suggest that the elevation in serum IGF-I concentrations achieved in the present study (less than 3-fold) was insufficient to induce measurable changes in fasting glucose kinetics or insulin sensitivity in nondiabetic subjects. It is probable that in diabetic subjects, high rates of hepatic glucose production and severely impaired peripheral glucose uptake are more responsive to the increase in serum IGF-I levels than the relatively normal HGP and glucose uptake rates in our nondiabetic subjects. Although there were no significant differences between IGF-I and placebo in the present study, insulin sensitivity shifted slightly in the "expected" (ie, greater insulin sensitivity) direction in the IGF-I group, hinting that physiologically relevant changes might have been apparent with a larger increase in plasma IGF-I.

If there is a dose-response relationship between the magnitude of change in plasma IGF-I concentrations and increased insulin sensitivity, a positive response could be observed in those subjects with the largest increase in IGF-I. When we examined the relationship between the change in plasma IGF-I concentration and alterations in plasma insulin concentrations or insulin sensitivity, the correlation coefficients were very low ($r^2 < .20$) however. Even the 2 subjects who did respond to treatment with > 4-fold increase in plasma IGF-I levels did not show dramatic changes in insulin sensitivity. In these subjects however, the final plasma IGF-I concentrations were still less than 350 ng/mL, considerably lower than the mean values in the prior studies on nondiabetic subjects. The possibility that the IGF-I dose used did not elevate plasma IGF-I concentrations above a "threshold" required to drive physiologically relevant changes in glucose kinetics or insulin sensitivity is a viable explanation for the observed results. Cusi and DeFronzo[2] reported that 7 days of IGF-I injection (increasing plasma IGF-I levels 3-fold) in patients with T2DM significantly reduced fasting glucose concentration and HGP, but the postprandial glucose response was unaffected by treatment. In contrast with the lack of change in insulin sensitivity we observed, protein synthesis was increased,[17] and lean tissue was added[18] using an IGF-I dose and route of administration identical to ours in a very similar study population. These data, in conjunction with other results[3-5] suggest that the quantitative impact of IGF-I treatment on intermediary metabolism is tissue and/or pathway-specific.

There also may be a diminution of the IGF-I effect over time. In prior studies, outcome variables were assessed after a shorter period of time; a few hours[3,4] or 2 to 7 days[2,5,6,9] except for the

aborted clinical trial in T2DM.[10] Thompson et al[18] observed that plasma IGF-I levels peaked after 2 days (at about 500 ng/mL) and then declined to about 300 ng/mL after 28 days of 30 μg/kg/d IGF-I in older women. Although lean body mass increased and fat mass decreased after the 28-day protocol, nitrogen retention declined back to baseline in the last week of that study. In a parallel study to the present one using many of the same subjects and an identical IGF-I dose and mode of delivery, we showed that IGF-I supplementation (posttreatment IGF-I concentration ~300 ng/mL) did not change body composition, bone density, or muscle strength after 6 months or 1 year relative to baseline.[25] In addition, Lieberman et al[30] reported that the anabolic actions of continuous intravenous IGF-I treatment in patients with human immunodeficiency virus (HIV) were ephemeral and nonexistent by day 10 of supplementation. Therefore, the data suggest that long-term supplementation with IGF-I causes tachyphylaxis that attenuates the physiologic response. It is possible that if we had repeated our baseline measurements after 5 to 7 days rather than after 4 weeks, we would have seen the expected enhancement in sensitivity to insulin. If so, increasing the dose of IGF-I treatment over time or administering it intermittently may be necessary to maintain any beneficial effect.

Finally, prior studies of IGF-I supplementation and insulin-mediated glucose metabolism were conducted in young adults who were in their twenties[3,5,6] or individuals with T2DM who were generally less than 50 years old.[2,4,9,10] The subjects in the current investigation were all > 60 years of age and postmenopausal. It is possible that the changes in plasma concentrations of IGF-I exert less influence on parameters related to glucose and insulin metabolism in older adults, although a mechanism to explain that difference is not obvious. If it were not age per se, but menopausal status (eg, presence or absence of estrogen and/or progesterone) that mediates the efficacy of IGF-I, we would expect to observe different responses in subjects on or off ERT.

Influence of HRT

The current study was not designed to directly test for potential differences between women on or off HRT, and results should be interpreted with some caution. Given the recent controversy regarding the potential impact of HRT on risk for cardiovascular disease, however, some of the data collected in the current study may be instructive. At baseline, the major outcome variables related to blood glucose kinetics and insulin sensitivity were similar in the 15 women using HRT and the 11 women who were not. The fasting blood glucose concentration was slightly lower in the HRT group, but this "difference" is not likely to have much clinical relevance because the actual group means (HRT group = 87 mg/dL, non-HRT group = 91 mg/dL) are similar and solidly in the normal fasting range. Prior data with respect to the influence of HRT on insulin sensitivity have been contradictory. Although there are some data showing that HRT enhances insulin sensitivity in women with T2DM,[31] results from nondiabetic women suggest that ERT either has no effect[22,32] or impairs insulin sensitivity.[23,24] In by far the largest study to date, cross-sectional analysis in thousands of women indicated no effects of HRT on glucose or insulin concentrations.[32]

We found that a greater portion of energy expenditure both before and during the clamp was derived from carbohydrate oxidation in the HRT group. Recently, Campbell and Febbraio[33] reported that glucose uptake was impaired in ovariectomized rats and restored by estrogen replacement. However, because there was no difference in blood glucose uptake between groups in the present study, the data imply that in the HRT group, a greater portion of glucose uptake was directed to oxidation rather than storage. If it is a real effect, the shift toward increased carbohydrate oxidation with HRT could have physiologic relevance. Low rates of fatty acid oxidation may increase lipid storage and have been implicated in the development of insulin resistance.[34,35] It is possible that the shift toward greater carbohydrate oxidation presages development of insulin resistance and may be connected to the adverse cardiovascular effects recently noted in large clinical trials of HRT.[36] At present, that argument is speculative and solid data derived from carefully controlled longitudinal studies will be required to address the issue directly.

The response to IGF-I administration did not vary with HRT status for any of the measured parameters. Based on data from the current study, there do not appear to be any interactions between circulating estrogen/progesterone and IGF-I that have regulatory effects on glucose and insulin metabolism. In the parallel long-term study, we also found no interactions between HRT use and IGF-I on body composition, muscle strength, and bone density.[25]

Potential Confounding Variables

Investigators have consistently shown that changes in energy balance, diet composition, and physical activity can have a profound influence on insulin sensitivity. In the present study, diet composition and energy content was rigidly controlled by requiring subjects to consume the same foods in the same quantities every day for 14 days before each testing period. Subjects were weighed every 2 days and energy content of the diet was adjusted if any deviations in body weight occurred. This aspect of the study design was successful in that body weight was maintained in both groups, eliminating changes in energy balance and nutrient composition as potential confounding variables. Control of physical activity was more difficult and depended to a greater extent on subject compliance. Subjects were instructed to maintain their usual patterns of physical activity and to refrain from exercise in the 2 days before the clamps. All subjects reported compliance with those instructions, but we have no independent verification. Because most subjects (but not all) were relatively sedentary, any systematic change in their patterns of physical activity would be in the direction of increased activity, which would be expected to enhance insulin sensitivity. As we observed no increase in sensitivity in either group, it is unlikely that subjects were becoming more physically active during the study in a way that would materially affect the results.

It is possible that the techniques used were not optimal to detect changes in fasting glucose metabolism or sensitivity to insulin. The stable isotope dilution method is the gold standard to measure whole-body glucose kinetics, however, and other investigators have noted changes with high-dose IGF-I treat-

ment using that technique.[2,4,5] The modified hyperglycemic clamp used was designed to modestly increase plasma glucose and insulin to typical postmeal concentrations. This goal was achieved and thereby, we hoped to partially suppress HGP, allowing us to directly assess the effects of IGF-I on hepatic insulin sensitivity. Unfortunately, HGP was suppressed to a much greater extent than was anticipated (100% in more than half of the subjects) in the baseline conditions, so the effects of IGF-I on suppression of HGP could not be accurately assessed. It is conceivable that maintaining a higher target plasma glucose concentration and inducing a greater stimulation of glucose uptake would have made any effects of IGF-I on whole-body insulin sensitivity more apparent. However, there was no pattern suggesting that IGF-I was having a measurable effect even in the most insulin-sensitive subjects, who responded to the clamp with impressive upregulation of plasma glucose uptake.

Clinical Applications

In addition to its well-known utility to induce anabolic responses in individuals who are insensitive to growth hormone, IGF-I has been viewed as a potentially beneficial therapy to combat the loss of lean tissue, gain of fat, and erosion of insulin sensitivity that often accompanies aging.[2,11,37] Although in short-term studies (hours to days), high-dose IGF-I administration induces positive effects on carbohydrate metabolism,[2-6,9,10] results from our 28-day study suggest that those same effects are not induced by low-dose subcutaneous injection and/or are not maintained over time. It is possible that a higher dose, a different mode of administration, and/or intermittent use of IGF-I will be necessary to prevent tachyphylaxis and allow any short-term effects to be sustained, although any benefits will have to be weighed against the real possibility of serious adverse reactions.

ACKNOWLEDGMENT

The authors express their deep appreciation for the considerable time and effort expended by the subjects in this study. We also acknowledge the expert assistance from Drs Scott Lee and Dwight Matthias, Jeanine Grillo, MS, RD, Sharon Moynihan, MS, RD, Jane Borchers MS, RD, Leah Holloway, BS, and the nursing staff at the Aging Studies Unit, Palo Alto VA Health Care System. We thank Genentech, for providing the rhIGF-I and placebo solutions and Diagnostic Systems Laboratories for the insulin and IGF-I radioimmunoassay kits.

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