Original Article

Effects of Recombinant Human IGF-I/IGF-Binding Protein-3 Complex on Glucose and Glycerol Metabolism in Type 1 Diabetes

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Recombinant human IGF-I (rhIGF-I) complexed with its natural binding protein IGF-binding protein (IGFBP)-3 (rhIGF-I/IGFBP-3) is a novel formulation that has been shown to improve insulin sensitivity in type 1 diabetes, yet the mechanisms are not clear. We used stable isotopes to investigate the effects of rhIGF-I/IGFBP-3 on glucose and glycerol metabolism in type 1 diabetes. Fifteen subjects (age 13–24 years; 10 males) were studied on three occasions in random order. Each study period lasted for two days, and an injection of either placebo or rhIGF-I/IGFBP-3 (0.1–0.8 mg · kg^{-1} · day $^{-1}$) was given subcutaneously at 6:00 P.M. on days 1 and 2. Following the second injection, the subjects were kept euglycemic overnight by a variable rate insulin infusion, followed by a 4-h, two-step (insulin 0.6 and 1.5 mU · kg^{-1} · min $^{-1}$) hyperinsulinemic-euglycemic clamp. During the overnight basal steady state, rhIGF-I/IGFBP-3 dose-dependently reduced endogenous glucose production rate (R_a) ($P = 0.004$), while peripheral glucose uptake (R_d) was not different from placebo. The increase in glucose R_d during hyperinsulinemic clamp was greater following rhIGF-I/IGFBP-3 than placebo, both during the first ($P = 0.008$) and second step ($P = 0.008$) of the clamp. No significant differences were found in glycerol R_a, a measure of lipolysis, between rhIGF-I/IGFBP-3 and placebo. In conclusion, rhIGF-I/IGFBP-3 enhances glucose metabolism by controlling both endogenous glucose output and peripheral glucose uptake. *Diabetes* 55:2365–2370, 2006

IGF-I enhances insulin sensitivity in subjects with and without diabetes (1,2), and administration of physiological doses of recombinant human IGF-I (rhIGF-I) improves glycemic control in young subjects with type 1 diabetes (3). However, the clinical use of rhIGF-I has been questioned due to a dose-dependent increase in side effects, most notably progression of retinal changes, associated with higher doses used in clinical trials (4).

The combination of rhIGF-I with IGF-binding protein (IGFBP)-3 prolongs the half-life of IGF-I in the circulation and may avoid the toxicity associated with free IGF-I (5) while providing similar efficacy in terms of insulin sensitivity. In adult patients with type 1 diabetes, Clemmons et al. (6) showed that a 2-week subcutaneous infusion of rhIGF-I/IGFBP-3 led to significant reductions in insulin doses and mean serum glucose levels without any side effects. We have recently demonstrated that a 2-day course of rhIGF-I/IGFBP-3, given as a subcutaneous injection at 6 P.M. to adolescents and young adults with type 1 diabetes, resulted in steady serum IGF-I levels for at least 14 h and significantly suppressed overnight growth hormone mean concentrations and pulse amplitude (7). This was paralleled by reduced overnight insulin requirements.

However, it has not been clear whether the effects of rhIGF-I/IGFBP-3 on insulin requirements in subjects with type 1 diabetes are mediated by decreased growth hormone secretion or by direct effects of IGF-I. Deleterious effects of growth hormone on glucose metabolism and insulin action are well documented, and various interventions to reduce growth hormone secretion in type 1 diabetes have been associated with reduced overnight insulin requirements (8,9). This may result from either direct effects of reduced growth hormone concentrations on suppression of hepatic glucose production and stimulation of periphral glucose uptake or by diminished lipolysis, reflected by reduced glycerol production rate and a decrease in circulating free fatty acids and ketones. IGF-I, on the other hand, may have direct insulin-like effects on hepatic glucose production and peripheral glucose uptake that are independent of its suppressive effects on growth hormone secretion (10).

To explore the mechanism of action of rhIGF-I/IGFBP-3 further, we have evaluated the effects on glucose production, glucose uptake, and glycerol turnover in subjects where we have previously reported improvements in overall insulin sensitivity as assessed by the M values (7). These further studies support a role for IGF-I in regulation of insulin sensitivity both directly and indirectly through suppression of growth hormone.

RESEARCH DESIGN AND METHODS

Eighteen adolescents and young adults with type 1 diabetes were recruited to the study. Fifteen subjects (10 males and 5 females, age range 13–24 years) completed the study protocol, and their data were used in the present

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IGFBP, IGF-binding protein; rhIGF-I, recombinant human IGF-I; NEFA, nonesterified fatty acid.

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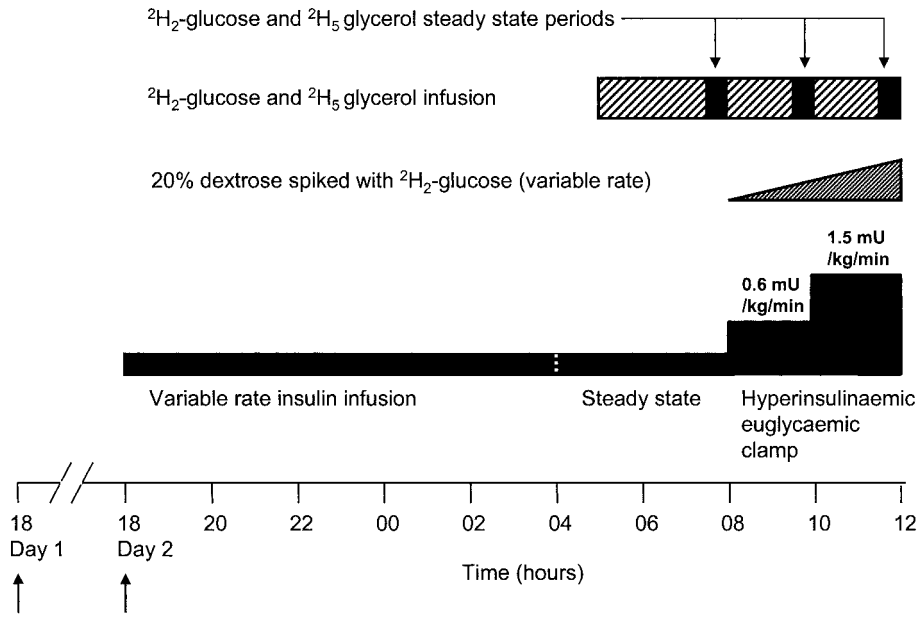

FIG. 1. Schematic representation of the overnight study protocol.

analysis. Inclusion criteria were type 1 diabetes of at least 2 years' duration or C-peptide negative, age between 13 and 25 years, puberty (Tanner) stage ≥II, treatment with two or more insulin injections per day, and normal renal and liver function. Exclusion criteria were BMI >30 kg/m^2, untreated hypothyroidism, chronic illness, pregnancy, malignancy, or recurrent episodes of severe unexplained hypoglycemia.

The study was approved by the Cambridge and Northampton Local Research Ethics Committees, and written informed consent was obtained from all subjects and/or from their parents. All overnight studies were performed in Cambridge in the Addenbrooke's Clinical Research Centre Wellcome Trust Clinical Research Facility.

This was a double-blind cross-over study in which subjects were randomly allocated to receive one of two study regimens (groups A and B). Treatment regimens in each group were comprised by the following. Group A (n = 7): *1*) rhIGF-I/IGFBP-3 complex (SomatoKin; Insmed, Richmond, VA) 0.1 mg/kg (equivalent of rhIGF-I 0.02 mg/kg), *2*) rhIGF-I/IGFBP-3 complex 0.4 mg/kg (rhIGF-I 0.08 mg/kg), and *3*) placebo, in random order. Group B (n = 8): *1*) rhIGF-I/IGFBP-3 complex 0.2 mg/kg (rhIGF-I 0.04 mg/kg), *2*) rhIGF-I/IGFBP-3 complex 0.8 mg/kg (rhIGF-I 0.16 mg/kg), and *3*) placebo, in random order. In each patient in each group, the study medications were administered for 2 days at 6:00 P.M. on three occasions separated by a washout period of at least 2 weeks. There were no differences in age, duration of diabetes, C-peptide level, HbA_{1c}, insulin dose, BMI, or baseline serum IGF-I levels between groups A and B (7).

Study protocol. Long- and intermediate-acting insulin was withdrawn and substituted with regular soluble insulin injections 36 h before the study start. The study medications were given as subcutaneous injections into the anterior aspect of the left thigh at 6.00 P.M. on days 1 and 2. After the second injection, the subjects were admitted to the investigation unit until 2:00 P.M. the next day. An intravenous insulin infusion was administered overnight (6:00 P.M. to 8:00 A.M.) to achieve euglycemia. Glucose levels were maintained around 5 mmol/l throughout the night by adjusting the intravenous insulin infusion based on 15-min blood glucose level measurements. The overnight steady-state period was defined as the period (4:00–8:00 A.M.) during which blood glucose levels did not differ significantly from 5 mmol/l. Insulin sensitivity was assessed as insulin requirement for euglycemia during the steady-state period (7) (Fig. 1).

At 5:00 A.M., primed infusions of glucose ([6,6-$^2H_2$] glucose, 170 mg intravenous bolus followed by continuous infusion 1.7 mg/min) and glycerol ($^2H_5$ glycerol, 0.61 mg/kg bolus followed by continuous infusion 0.15 mg · kg^{-1} · min^{-1}) were commenced and continued for the duration of the study.

A 4-h, two-step hyperinsulinemic-euglycemic clamp was performed from 8:00 A.M. to 12:00 P.M. Between 8:00 and 10:00 A.M., a low-dose insulin infusion (insulin bolus 2.8 mU/kg, infusion 0.6 mu · kg^{-1} · min^{-1}) was given and euglycemia maintained by an infusion of 20% dextrose spiked with 7 mg/g [6,6-$^2H_2$] glucose to maintain steady enrichment values. Between 10:00 A.M. and 12:00 P.M., the insulin infusion rate was increased (insulin bolus 7.0 mU/kg, infusion 1.5 mU · kg^{-1} · min^{-1}) and the infusion of 20% dextrose, spiked with 7 mg/g [6,6-$^2H_2$] glucose, increased as necessary to maintain euglycemia. Blood glucose measurements were performed every 5 min.

Biochemical analyses. Blood glucose concentrations were measured using 25-μl whole-blood samples on a Y.S.I. model 2300 stat plus analyzer (YSI; Lynchford House, Farnborough, Hants, U.K.). Plasma insulin concentrations were measured using an enzyme-linked immunosorbent assay (DAKO; Denmark House, Angel Drove, Ely, Cambs, U.K.) according to the manufacturer's instructions. Plasma IGF-I, IGFBP-1, and IGFBP-3 levels were measured using an enzyme-linked immunosorbent assay (DSL/Oxford Bio-Innovations, Upper Heyford, Oxon, U.K.) according to the manufacturer's instructions. β-Hydroxybutyrate was measured using a standard enzymatic technique (β-Hydroxybutyric acid (NEFA) kit; Sigma Diagnostics, Poole, Dorset, U.K.). Nonesterified fatty acid (NEFA) concentrations were measured using a commercial kit (Half Micro test; Boehringer Mannheim, Lewes, U.K.). Glycerol concentration was measured using a commercially available colorimetric assay (Randox Laboratories, Antrim, U.K.) on an automated analyser.

Glucose and glycerol enrichments were measured by gas chromatography–mass spectrometry using a Hewlett-Packard 5971A MSD (Agilent Technologies, Stockport, U.K.), as described in detail elsewhere (11,12).

Calculations. Overnight steady-state insulin requirement for euglycemia was defined as mean insulin infusion rate (mU · kg fat-free body mass^{-1} · min^{-1}) The enrichments of glucose and glycerol were expressed as the tracer-to-tracee ratio. The rates of appearance and uptake of glucose (glucose R_a and R_d, respectively) and the rate of appearance of glycerol (glycerol R_a) were calculated using the Steele model for the nonsteady state modified for use with stable isotopes (13,14). The calculation was also modified for inclusion of [6,6-$^2H_2$] glucose in the dextrose infusion (15). Before calculation of glucose turnover, plasma glucose concentration and glucose enrichment time courses were smoothed using optimal segments technique analysis (16). The following steady-state periods were used for the calculations: overnight steady-state period, 7:30–8:00 A.M.; step one of the hyperinsulinemic clamp, 9:30–10:00 A.M.; and step two of the clamp, 11:30 A.M. to 12:00 P.M. The effective volume of distribution was assumed to be 143 ml/kg for glucose and 230 ml/kg for glycerol. In the basal state, glucose and glycerol production are inversely related to insulin concentration and therefore their rates of appearance are expressed as [glucose R_a (μmol · kg^{-1} · min^{-1}) × insulin concentration (mU/l)] and [glycerol R_a (μmol · kg^{-1} · min^{-1}) × insulin concentration (mU/l)], respectively. Glucose uptake in the basal state is directly related to insulin concentration, and therefore the rate of glucose uptake is expressed as [glucose R_d (μmol · kg^{-1} · min^{-1}) × insulin concentration (mU/l)$^{-1}$]. Mean glycerol concentrations were calculated for the three steady-state periods (7:30-8:00 A.M., 9:30–10:00 A.M., and 11:30 A.M. to 12:00 P.M.). For NEFA and β-hydroxybutyrate concentrations, area under curve was calculated for the overnight euglycemic steady state (4:00–8:00 A.M.).

Statistical methods. All statistical analyses were performed on paired data, with the placebo period serving as the control for each subject. The between-dose effects were studied using ANCOVA controlling for repeated assessments on same individual. Post hoc analysis was made by paired t tests with Bonferroni correction for multiple comparisons. Distributions of the data were examined for normality by the Kolmogorov-Smirnov goodness-of-fit test. Data that were not normally distributed (glucose R_a glucose R_d, glycerol R_a,

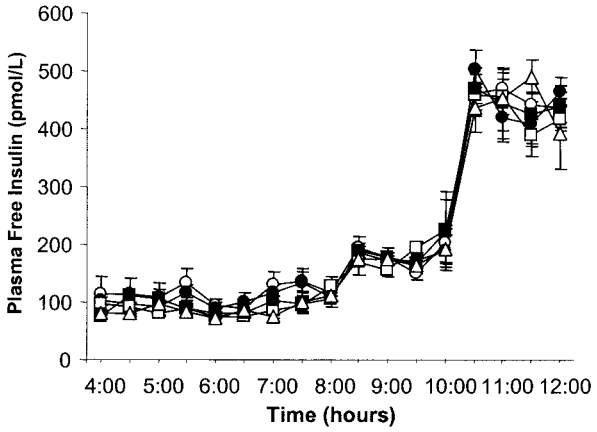

FIG. 2. Plasma insulin concentrations throughout the overnight variable rate insulin infusion euglycemic steady state and the two-step hyperinsulinemic clamp. ○, placebo; ●, rhIGF-I/IGFBP-3 0.1 mg · kg^{-1} · day^{-1}; □, 0.2 mg · kg^{-1} · day^{-1}; ■, 0.4 mg · kg^{-1} · day^{-1}; △, 0.8 mg · kg^{-1} · day^{-1}. Data are means ± SE.

NEFA, and β-hydroxybutyrate concentrations) were logarithmically transformed before statistical analysis. All statistical analyses were performed using SPSS for Windows (version 11.0; SPSS, Chicago, IL).

RESULTS

Glucose metabolism. Serum free insulin concentrations during the overnight euglycemic steady state and the hyperinsulinemic clamp are shown in Fig. 2. There were no significant differences in free insulin concentration between treatments at any stage of the study. We have previously reported significant dose-dependent reductions in overnight insulin requirements for euglycemia following IGF-I/IGFBP-3, paralleled by reductions in overnight growth hormone secretion (7). Basal endogenous glucose production rate (glucose R_a) following overnight euglycemic steady state was dose-dependently decreased following IGF-I/IGFBP-3 (P = 0.004, ANCOVA) (Fig. 3*A*). Peripheral glucose uptake (glucose R_d) was not changed in the basal state (P = 0.25; Fig. 3*B*).

In our earlier analysis, we found that insulin sensitivity assessed by the two-step hyperinsulinemic-euglycemic clamp, defined by M values, was increased following the two highest doses of rhIGF-I/IGFBP-3 (0.4 and 0.8 mg · kg^{-1} · day^{-1}), whereas the lower doses (0.1 and 0.2 mg · kg^{-1} · day^{-1}) had little effect on insulin sensitivity. Here we analyzed the changes in glucose metabolism. Endogenous glucose production was reduced during the clamp, but there was no difference between placebo and rhIGF-I/IGFBP-3 (Fig. 4*A*). The increase in peripheral glucose uptake during hyperinsulinemic clamp, on the other hand, was greater following rhIGF-I/IGFBP-3 than placebo, during both the first (P = 0.008, ANCOVA) and second step (P = 0.008) of the clamp (Fig. 4*B*).

Lipid metabolism. Basal glycerol concentrations were similar following placebo and rhIGF-I/IGFBP-3 (Table 1). The basal endogenous glycerol R_a during overnight euglycemic steady state tended to be decreased following rhIGF-I/IGFBP-3 (Fig. 3*C*), but this was not statistically significant (P = 0.08, ANCOVA). There were no significant changes in area under curve of NEFA or β-hydroxybutyrate concentrations, adjusted for insulin concentration, during the overnight steady-state period following either placebo or IGF-I/IGFBP-3 (Table 1). During the hyperinsulinemic clamp, glycerol R_a was decreased following placebo and rhIGF-I/IGFBP-3, with no difference between treatments (Fig. 4*C*). NEFA, β-hydroxybutyrate, and glycerol concentrations were also reduced during the clamp, but the reductions were similar following placebo and rhIGF-I/IGFBP-3 (data not shown).

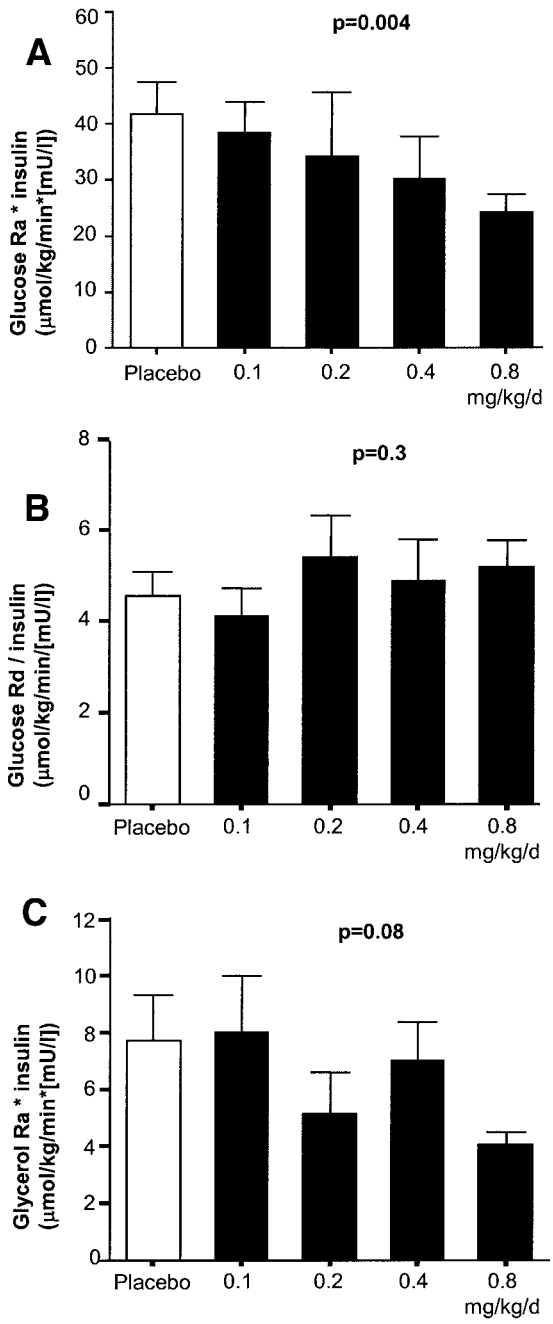

FIG. 3. Endogenous glucose production (*A*; glucose R_a), peripheral glucose uptake (*B*; glucose, R_d), and endogenous glycerol production (*C*; glycerol R_a) during overnight euglycemic steady state (7:30–8:00 A.M.) following 2-day treatment of rhIGF-I/IGFBP-3, corrected for plasma insulin concentration. Data are means ± SE.

Sex differences. There were no differences between sexes in endogenous glucose production, peripheral glucose uptake, glycerol production rate, NEFA, or β-hydroxybutyrate concentrations or in the effect of rhIGF-I/IGFBP-3 on these parameters (data not shown).

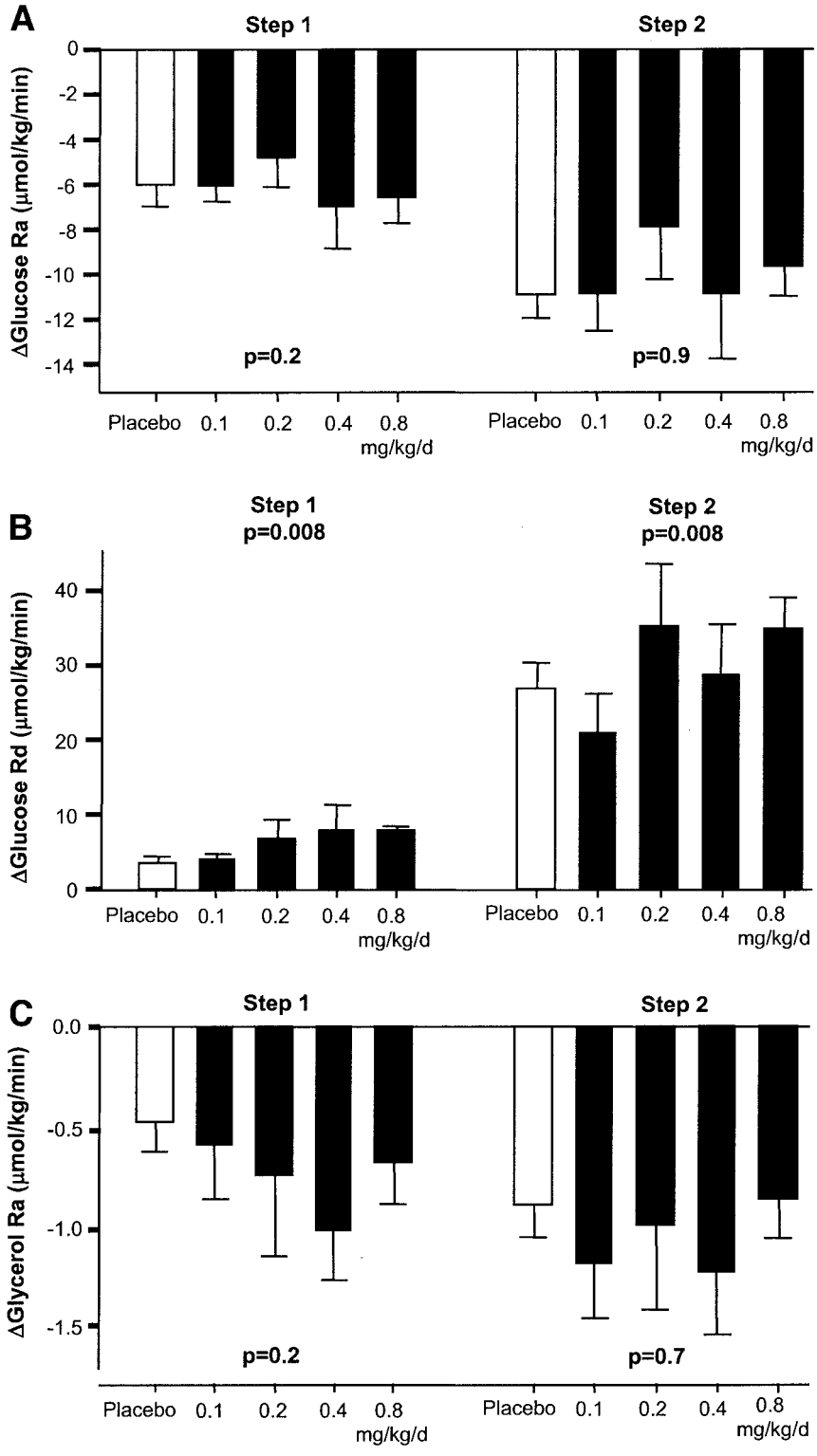

FIG. 4. Changes from baseline in endogenous glucose production (*A*; glucose R_a), peripheral glucose uptake (*B*; glucose R_d), and endogenous glycerol production (*C*; glycerol R_a) during a two-step hyperinsulinemic-euglycemic clamp following 2-day treatment of rhIGF-I/IGFBP-3. *Left panel*: step 1: insulin 0.6 mU · kg^{-1} · min^{-1}, from 8:00–10:00 A.M., steady-state 9:30–10:00 A.M. *Right panel*: step 2: insulin 1.5 mU · kg^{-1} · min^{-1}, from 10:00 A.M. to 12:00 P.M., steady-state from 11:30 A.M. to 12:00 P.M. Data are means ± SE.

DISCUSSION

This study addressed the effects of a 2-day course of a combination of recombinant IGF-I and IGFBP-3 on glucose and lipid metabolism in adolescents and young adults with type 1 diabetes. Our results show that reduced overnight insulin requirements following rhIGF-I/IGFBP-3 are associated with suppressed endogenous glucose production, whereas improved insulin sensitivity determined by hyperinsulinemic clamp is related to an increased rate of peripheral glucose uptake.

Our finding that endogeneous glucose production was reduced during the overnight study yet peripheral glucose uptake was not increased suggests that suppression of nocturnal growth hormone secretion may be a key mechanism whereby rhIGF-I/IGFBP-3 reduces insulin requirements for euglycemia overnight. This is in accordance with our finding of a dose-dependent reduction of up to 61% in mean overnight growth hormone levels following rhIGF-I/IGFBP-3 (7). Earlier studies have shown that increased pulsatile growth hormone secretion markedly

stimulates hepatic glucose production in subjects with type 1 diabetes (17,18), whereas suppression of growth hormone secretion by somatostatin abolished the increase in endogenous glucose production (18). A direct effect of rhIGF-I/IGFBP-3 on hepatic glucose production might also be speculated. Simpson et al. (10) recently demonstrated lower glucose R_a following a single injection of rhIGF-I 40 μg/kg in patients with type 1 diabetes when endogenous growth hormone secretion was completely suppressed by octreotide, therefore suggesting some direct effect of IGF-I on glucose R_a. However, the mechanism for this is unclear since it has been reported that there are a paucity of IGF-I receptors in the liver (19). Earlier studies of subjects with type 1 diabetes receiving recombinant IGF-I failed to show any effect on glucose R_a in basal state (20,21). Yet to conclusively exclude any hepatic effect of rhIGF-I/IGFBP-3, suppressed growth hormone secretion should be replaced by exogenous growth hormone administration. The fact that we did not find any improvement by rhIGF-I/IGFBP-3 in glucose R_d in the overnight study may be explained by the fact that in the basal state, the effect of insulin on glucose metabolism is mainly on endogenous glucose output and to a much smaller degree on glucose uptake. Therefore, one would not expect to detect any substantial changes in glucose R_d. Thus we conclude that the effects of rhIGF-1/IGFBP-3 are largely explained by growth hormone suppression, but we cannot exclude other possible mechanisms such as direct effects on renal gluconeogenesis or changes in hepatic blood flow.

The hyperinsulinemic clamp study demonstrated a dose-dependent effect of rhIGF-I/IGFBP-3 on glucose R_d in the hyperinsulinemic state. This indicates that improvement in insulin sensitivity by rhIGF-I/IGFBP-3 is related to direct effects of IGF-I on peripheral glucose uptake. Suppression of growth hormone probably does not play a major role here, since rhIGF-I/IGFBP-3 had no effect on the reductions in glucose R_a during the clamp. However, because hepatic glucose production is strongly suppressed by hyperinsulinemia per se, subtle changes in glucose R_a by increased IGF-I levels or by suppressed growth hormone may be difficult to detect. The clamp protocol was performed immediately following the overnight study, commencing 14 h after the second injection of rhIGF-I/IGFBP-3. Our result of increased glucose R_d during the clamp is similar to that found in the study by Simpson et al. (10), in which the hyperinsulinemic clamp was started 5 h after the injection of rhIGF-I. In contrast, Acerini et al. (20) did not find any effect on glucose R_d when rhIGF-I was injected 14 h before the clamp. Therefore, our results suggest that the combination of rhIGF-I with rhIGFBP-3 provides significantly prolonged effect on glucose metabolism compared with rhIGF-I alone. Repeated administration of rhIGF-I may result in similarly sustained improvement of glucose metabolism, since a 7-day course of rhIGF-I (40 $\mu g \cdot kg^{-1} \cdot day^{-1}$) resulted in increased glucose R_d in a clamp performed 11 h after the last injection (21).

Studies on the effects of IGF-I on lipolysis in normal, growth hormone–deficient, and diabetic subjects have yielded conflicting results, reflecting the complex feedback regulation between growth hormone, IGF-I, and insulin and the resulting variance in the net effect on lipolysis following any intervention. According to the present study, the effect of rhIGF-I/IGFBP-3 on overnight insulin requirement and glucose R_a in type 1 diabetes does not seem to be mediated by changes in lipolysis or NEFA

TABLE 1
Plasma NEFA, β-hydroxybutyrate, and glycerol concentrations during the overnight euglycemic (90 ± 9 mg/dl) steady-state period (4:00–8:00 A.M.), after 2 days of rhIGF-I/IGFBP-3 complex or placebo given as subcutaneous injection at 6:00 P.M. on each day

	Group A (n = 7)			Group B (n = 8)		
Dose of rhIGF-I/IGFBP-3	Placebo	0.1 mg · kg^{-1} · day^{-1}	0.4 mg · kg^{-1} · day^{-1}	Placebo	0.2 mg · kg^{-1} · day^{-1}	0.8 mg · kg^{-1} · day^{-1}
NEFA AUC	1,500 ± 213	1,377 ± 189	1,514 ± 305	1,683 ± 206	1,688 ± 288	1,326 ± 215
β-hydroxybutyrate AUC	0.71 ± 0.14	0.77 ± 0.21	0.66 ± 0.23	1.16 ± 0.20	0.87 ± 0.19	0.95 ± 0.26
Glycerol (μmol/l)	54.6 ± 4.7	56.0 ± 7.6	52.8 ± 7.7	48.6 ± 8.3	52.4 ± 6.3	39.0 ± 7.1

Data are mean ± SE. $P > 0.05$ for all comparisons between placebo and rhIGF-I/IGFBP-3, adjusted for plasma insulin concentration. NEFA and β-hydroxybutyrate determined as area under curve (AUC) between 4:00 and 8:00 A.M. Glycerol concentration determined as mean of repeated samples between 7:30 and 8:00 A.M.

concentrations, since no significant differences in these parameters were noticed, except for a trend toward decreased basal glycerol R_a. Our results corroborate an earlier study showing no change in glycerol R_a in subjects with type 1 diabetes following rhIGF-I (10). In the hyperinsulinemic clamp study, suppression of glycerol R_a was not enhanced by rhIGF-I/IGFBP-3 and reductions in NEFA and glycerol concentrations were similar on placebo and rhIGF-I/IGFBP-3. Hyperinsulinemia is a strong inhibitor of lipolysis, whereas only very high doses of IGF-I seem to have any direct effect on lipolysis (22), possibly due to relatively low numbers of IGF-I receptors in adipocytes (23). Serum IGF-I levels in our subjects following rhIGF-I/IGFBP-3 administration were in the physiological range (7), and therefore we did not expect to see much effect on lipolysis under hyperinsulinemic conditions.

In summary, we have shown that the effects of the combination of rhIGF-I and rhIGFBP-3 on glucose metabolism in type 1 diabetes involve both direct and growth hormone–mediated mechanisms. In the basal state, endogenous glucose production is decreased in parallel to suppression of growth hormone secretion, whereas during hyperinsulinemia, rhIGF-I/IGFBP-3 directly increases peripheral glucose uptake. rhIGF-I/IGFBP-3 thus enhances both basal and insulin-stimulated glucose metabolism. Whether this can be translated into improved glycemic control in type 1 diabetes is to be demonstrated by long-term clinical trials.

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