Dose-Response Effect of a Single Administration of Oral Hexyl-Insulin Monoconjugate 2 in Healthy Nondiabetic Subjects

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OBJECTIVE — *1*) To evaluate the effect of a single oral dose of hexyl-insulin monoconjugate 2 (HIM2) on the rate of whole-body glucose disposal (R_d) and endogenous glucose production (EGP) in healthy nondiabetic subjects, *2*) to examine the reproducibility of HIM2 on glucose metabolism, and *3*) to compare the results obtained with HIM2 with those using a bioequivalent dose of subcutaneous lispro insulin.

RESEARCH DESIGN AND METHODS — Six healthy subjects ([means ± SE] aged 31 ± 5 years and BMI 23.1 ± 3.9 kg/m^2) participated in four studies performed in random order on separate days. Subjects ingested a single dose of HIM2 (0.125, 0.5, and 0.75 mg/kg) or received subcutaneous lispro insulin (0.1 units/kg). Studies were performed with [3-^{3}H]glucose, and plasma glucose concentration was maintained at basal levels for 4 h with the euglycemic clamp technique. After 6 weeks, subjects participated in two repeat studies to examine the reproducibility of HIM2 (0.5 mg/kg) and lispro insulin (0.1 units/kg).

RESULTS — Fasting plasma insulin (7 μU/ml) increased to a maximum of 102, 321, and 561 μU/ml at 60 min after all three HIM2 doses (0.125, 0.5, and 0.75 mg/kg, respectively). A dose-related decrease in basal EGP was observed as the HIM2 dosage was increased from 0 to 0.125 to 0.5 mg/kg ($P < 0.05$ vs. each preceding dose). Suppression of EGP was similar with the 0.5- and 0.75-mg/kg HIM2 doses. A dose-related stimulation of basal R_d was observed as the HIM2 dosage was increased from 0 to 0.125 to 0.5 ($P < 0.05$ vs. each preceding dose) to 0.75 mg/kg ($P < 0.10$ vs. preceding dose). R_d (0–240 min) was increased by 0.5 mg/kg oral HIM2 to a value similar to 0.1 units/kg lispro insulin. The 0.125-mg/kg HIM2 dose reduced EGP (0–240 min) to a value that was similar to 0.1 units/kg lispro insulin. The variability in the effect of HIM2 and lispro on R_d (25 ± 7 vs. 27 ± 1%, respectively) and on suppression of EGP (19 ± 1 vs. 19 ± 0.7%, respectively) was similar.

CONCLUSIONS — Oral HIM2 suppresses EGP and increases tissue R_d in a dose-dependent manner. The effects of HIM2 on EGP and R_d persisted at 240 min, even though plasma insulin concentration had returned to basal levels. Oral HIM2 may provide an effective and reproducible means of controlling postprandial plasma glucose excursions in diabetic patients.

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Abbreviations: EGP, endogenous glucose production; FFA, free fatty acid; HIM2, hexyl-insulin monoconjugate 2.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Insulin is secreted by the pancreas directly into the portal venous system. The liver clears approximately half of the insulin it receives, resulting in two- to threefold higher plasma insulin concentrations in the portal vein than in the peripheral circulation (1). Physiological control of postprandial glucose levels is achieved by simultaneous suppression of glucose production (primarily hepatic), increased hepatic glucose uptake, and stimulation of glucose utilization by peripheral tissues, primarily muscle (2–4). Because of the location of the liver, portal insulin delivery results in the effective suppression of hepatic glucose production without excessive peripheral hyperinsulinemia and augmented stimulation of glucose uptake by nonhepatic (primarily muscle) tissues. In diabetic patients, insulin is given peripherally, and systemic insulin concentrations are higher than in the portal vein (1). Consequently, glycemic control with conventionally administered insulin is exerted primarily by stimulation of glucose utilization in peripheral tissues, i.e., muscle and, to a lesser extent, adipose tissue (5). We (6) and others (7,8) have shown that peripheral hyperinsulinemia induces severe muscle insulin resistance. Inadequate hepatic insulinization results in reduced capacity of the liver to oxidize and store carbohydrate loads and impaired suppression of hepatic glucose production in diabetic patients (9–12). Based on this overview of normal physiology, it follows that the portal route of insulin administration is preferred over systemic administration.

The DCCT (Diabetes Control and Complications Trial) research group (13) and the UKPDS (U.K. Prospective Diabetes Study) group (14) have demonstrated that intensive treatment with insulin and/or oral agents reduces the incidence and rate of progression of microvascular complications in type 1 and type 2 diabetic patients, respectively. However, all current insulin replacement techniques produce transient peripheral hyperinsu-

Table 1—*EGP and whole-body tissue R_d during the euglycemic clamp studies performed with oral HIM2 and lispro insulin*

	Basal EGP	EGP (0–240 min)	R_d (0–240 min)
0.125 mg/kg HIM2	2.36 ± 0.11	1.13 ± 0.21	3.08 ± 0.22*
0.5 mg/kg HIM2	2.24 ± 0.20	0.76 ± 0.21	4.27 ± 0.35
0.75 mg/kg HIM2	2.18 ± 0.12	0.62 ± 0.12	5.10 ± 0.50
0.1 units/kg lispro insulin	2.31 ± 0.10	1.20 ± 0.51*	4.21 ± 0.41

Data are means ± SE. All values are presented as $mg \cdot kg^{-1} \cdot min^{-1}$. *$P < 0.05$ vs. 0.5- and 0.75-mg/kg doses of HIM2.

linemia, which is associated with increased incidence of hypoglycemia and weight gain (13,14), undesirable side effects that present obstacles to therapy. In addition, intensive insulin regimens rely on frequent injections of short-acting insulin to control meal-related glycemic excursions, and some diabetic patients find this inconvenient.

Hexyl-insulin monoconjugate 2 (HIM2) is a modified form of human insulin in which a single amphiphilic oligomer is covalently linked to the free amino acid group on the Lys-β29 residue of recombinant human insulin via an amide bond. This alters the physical-chemical characteristics, leading to enhanced stability and resistance to intestinal degradation of ingested insulin (15). Previous studies in type 1 and type 2 diabetic patients have shown that HIM2 is absorbed from the gastrointestinal tract in significant amounts and possesses hypoglycemic action (16,17). Because oral HIM2 is absorbed directly into the portal circulation, it may provide a more effective suppression of hepatic glucose output without producing peripheral hyperinsulinemia.

The primary aim of this study was to determine the dose-response effect of a single dose of HIM2 on endogenous (hepatic) glucose production (EGP) and the rate of glucose disposal (R_d) by peripheral (primarily muscle) tissues using the euglycemic clamp technique (18) in healthy nondiabetic subjects. We also examined the reproductibility of HIM2 administered to the same individual on separate days and compared the results with those of a bioequivalent dose of subcutaneous lispro insulin (19).

RESEARCH DESIGN AND METHODS

— Six healthy volunteers (two men and four women, aged 31 ± 5 years, weight 70 ± 8 kg, and BMI 23.1 ± 3.9 kg/m^2) participated in the study. Subjects had stable body weight for at least 6 months before the study, did not participate in heavy exercise on a regular basis, and were not taking any medications. Subjects gave informed written consent before participation. The protocol was approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio.

The study had a randomized, open-label, crossover design and consisted of the following three parts: *1*) a screening visit during which subjects underwent medical history, physical exam, routine blood tests, urinalysis, electrocardiogram, and an oral glucose tolerance test; *2*) three doses of oral HIM2 (0.125, 0.5, and 0.75 mg/kg, corresponding to 3.5, 14.4, and 21.5 units/kg, respectively) and one dose of lispro insulin (0.1 units/kg s.c.) in combination with tritiated glucose and euglycemic clamp technique; the four studies were performed in randomized order with a 3- to 7-day interval between each study; and *3*) after 6 weeks, repeat HIM2 (0.5 mg/kg) and lispro insulin (0.1 units/kg) studies were performed to evaluate intrasubject variability. HIM2 was administered as an oral liquid preparation containing the study drug and a diluent (total of 20 ml).

Studies were performed at the Clinical Research Center of the Texas Diabetes Institute at 7 A.M. following a 10-h overnight fast. After catheter insertion into an antecubital vein, the subjects received a prime (25 μCi) continuous (0.25 μCi/min) infusion of [3-^{3}H]glucose for 6 h. A second catheter was inserted retrogradely into a vein on the dorsum of the hand, which was then placed in a heated box (60°C) to arterialize venous blood. Baseline blood samples for determination of plasma [3-^{3}H]glucose radioactivity and plasma glucose and insulin concentrations were drawn at −30, −20, −10, −5, and 0 min, i.e., 90–120 min after starting of tritiated glucose. At time 0, subjects received a single oral dose of HIM2 (0.125, 0.5, or 0.75 mg/kg) or a subcutaneous lispro insulin injection (0.1 units/kg). Following oral HIM2 and lispro insulin, plasma glucose concentration was measured every 5 min, and a variable 20% dextrose infusion was adjusted based on the negative feedback principle to maintain euglycemia (18) for 240 min. Blood samples for measurement of plasma insulin concentration and [3-^{3}H]glucose radioactivity were collected every 15–20 min following insulin administration.

Analytical determinations

Plasma glucose was measured using the glucose oxidase method (Glucose Analyzer 2; Beckman Instruments, Fullerton, CA). Plasma insulin was determined using a radioimmunoassay kit specific for human insulin (Linco Research, St. Charles, MO), with a lower limit of detection of 0.07 ng/ml (2 μU/ml). Both regular and lispro insulin are equally recognized in this assay, and [3-^{3}H] glucose radioactivity was determined on barium/zinc deproteinized plasma samples.

Calculations

Under steady-state postabsorptive conditions, the rate of EGP equals the rate of whole-body R_d and was calculated as the [3-^{3}H]glucose infusion rate (desintegration per minute [DPM]) divided by the steady-state plasma [3-^{3}H]glucose-specific activity (DPM per milligram). Following insulin, non–steady-state conditions prevail and rates of glucose appearance (R_a) and R_d were calculated from Steele's equation (20), using a distribution volume of 250 ml/kg. EGP was calculated by subtracting the exogenous glucose infusion rate from the tracer-derived rate of R_a. Results are expressed in milligrams per kilogram per minute.

Statistical analysis

Statistical calculations were performed with StatView for Windows (version 5.0; SAS Institute, Cary, NC). Comparison between studies was made using repeated-measures ANOVA for specified time intervals, as indicated in the RESULTS section and in Table 1. All data are presented as

Table 2—*Mean plasma insulin concentration (μU/ml) during the euglycemic clamp after oral HIM2 and lispro insulin*

	HIM2			
	0.125 mg/kg	0.5 mg/kg	0.75 mg/kg	Lispro (0.1 units/kg)
0 min	7 ± 0.5	7 ± 0.3	7 ± 0.5	7 ± 0.2
60 min	102 ± 1	321 ± 7	560 ± 10	38 ± 5
120 min	18 ± 3	48 ± 7	108 ± 3	32 ± 2
180 min	9 ± 0.4	23 ± 1	30 ± 4	19 ± 1
240 min	6 ± 0.1	11 ± 0.3	13 ± 0.8	12 ± 0.5

Data are means ± SE in six subjects.

the mean ± SE. $P < 0.05$ was considered to be statistically significant.

RESULTS — All six subjects participated in all six studies. Oral HIM2 was well tolerated, and there were no changes in vital signs or side effects (including headache, dizziness, flushing, or adverse gastrointestinal/cardiovascular/pulmonary/dermatologic symptoms) reported by any subject during the study.

Fasting plasma insulin concentration (7.0 ± 0.4 μU/ml) increased to a maximum at 60 min after all three HIM2 doses (102, 321, and 560 μU/ml after 0.125-, 0.5-, and 0.75-mg/kg doses, respectively) and returned toward baseline shortly thereafter (Table 2). The plasma concentration time profile for lispro insulin was similar to that of HIM2, although the absolute plasma insulin concentrations achieved were significantly less ($P < 0.01$) than with HIM2 from 0 to 120 min.

All oral doses of HIM2 significantly ($P < 0.05$) reduced EGP compared with baseline, with a nadir between 30 and 90 min and a gradual return toward baseline thereafter (Fig. 1*A*). Suppression of EGP was similar ($P > 0.70$) during 0.5- and 0.75-mg/kg doses of HIM2 at all time intervals. Suppression of EGP with a 0.125-mg/kg dose of HIM2 was significantly less ($P < 0.05$) than with 0.5- and 0.75-mg/kg HIM2 doses after the 0- to 60-min time period. The suppression of EGP with lispro insulin (0.1 units/kg) from 0 to 60 min was significantly ($P = 0.01$) less than with all three HIM2 doses. Thereafter, the suppression of EGP with lispro insulin was similar to the 0.125-mg/kg HIM2 dose and significantly less than both the 0.05- and 0.75-mg/kg HIM2 doses (Fig. 1*A*).

With each increasing dose of HIM2, there was a progressive increase in R_d over the 0- to 240-min time period (Fig. 1*B*). The increases in R_d with both the 0.5- and 0.75-mg/kg HIM2 doses were significantly greater ($P < 0.05$) than with the 0.125-mg/kg HIM2 dose of all time intervals. From 60 to 180 min, the increase in R_d with the 0.75-mg/kg HIM2 dose was significantly greater than ($P < 0.05$) with the 0.5-mg/kg dose. During the 0- to 60-min and 180- to 240-min time intervals, R_d was similarly increased ($P > 0.70$) with both the 0.75- and 0.5-mg/kg HIM2 doses (Fig. 1). After 60 min, the stimulatory effect of 0.1 units/kg lispro insulin was similar to the 0.5- and 0.75-mg/kg HIM2 doses ($P > 0.70$ vs. both) (Fig. 1*B*).

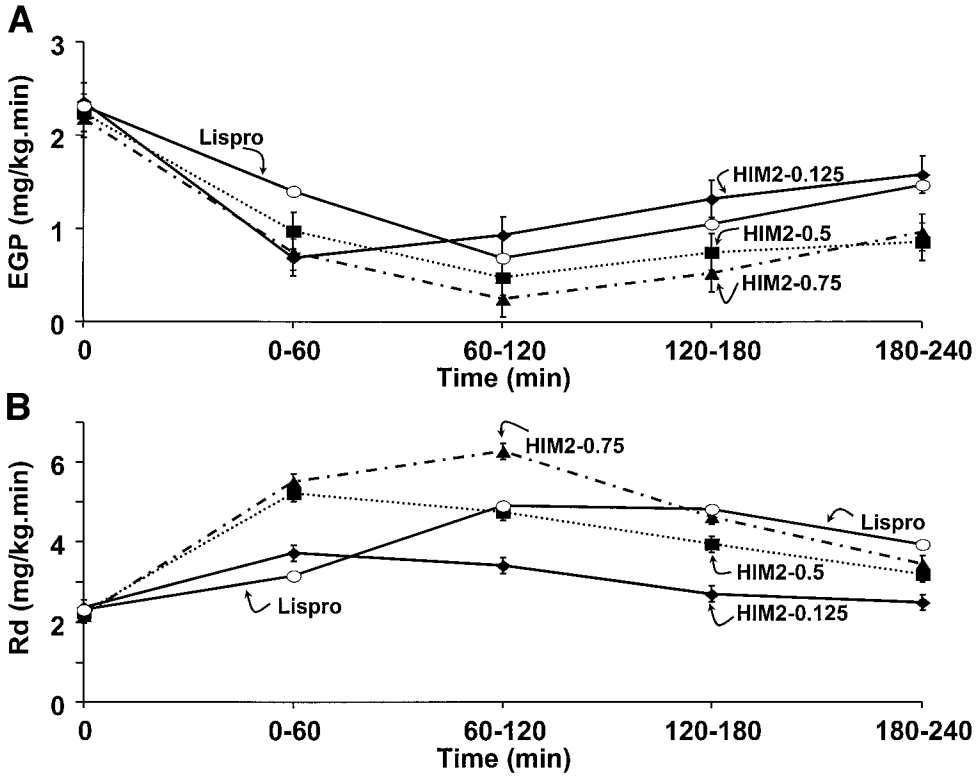

Figure 1—*Dose-response effect of HIM2 on suppression of EGP (A) and stimulation of tissue R_d (B) during the euglycemic clamp in all six subjects.*

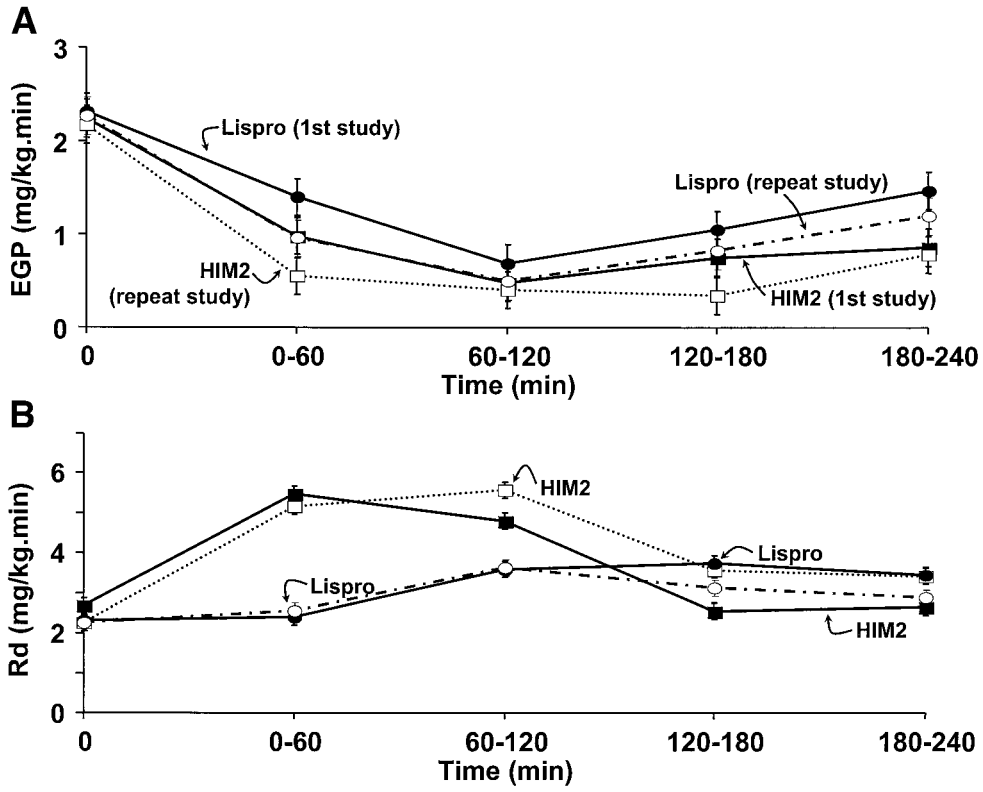

Figure 2—*Reproducibility of oral HIM2 (0.5 mg/kg) and subcutaneous lispro insulin (0.1 units/kg) on the suppression of EGP* (A) *and stimulation of tissue* R_d (B) *during the euglycemic clamp in all six subjects. Each subject participated in a repeat study ~6 weeks after the first study.*

The mean data for R_d and EGP for the repeat studies performed with the 0.5-mg/kg dose of HIM2 and 0.1 units/kg of lispro are shown in Fig. 2. The variability in the effect of HIM2 and lispro on R_d was 25 ± 2 and 27 ± 1%, respectively. The variability in the effect of HIM2 and lispro on the suppression of EGP was 19 ± 1 vs. 19 ± 1%, respectively.

CONCLUSIONS — In the present study, we examined the dose-response effect of oral insulin HIM2 on the suppression of EGP (hepatic) and peripheral tissue (muscle) R_d in healthy subjects using the euglycemic clamp technique. Our results demonstrate that oral HIM2 is rapidly absorbed from the gastrointestinal tract, with peak plasma insulin concentrations reached within 60 min and returning to baseline after 2 h. HIM2 caused a dose-dependent suppression of EGP and a dose-dependent stimulation of R_a, with maximum effects being observed with 0.5 mg/kg HIM2 (Table 1). It is noteworthy that with all doses of HIM2 (0.125–0.75 mg/kg), EGP after 240 min remained significantly below baseline values, while R_d remained significantly elevated (Fig. 1 and Table 1) even though the plasma insulin concentration had returned to fasting levels (Table 2).

The explanation for the persistent effect of HIM2 on peripheral tissues and the liver remains to be elucidated. It is possible that the pharmacokinetic interaction (i.e., increased binding affinity and/or capacity) of HIM2 with the insulin receptor in muscle and liver differs from that of native insulin, resulting in a more prolonged activation of the insulin receptor. This would also explain the more rapid clearance of HIM2 compared with lispro insulin (Table 2) from the peripheral circulation. A similar prolongation of the biologic action of HIM2 after the plasma insulin concentration returns to baseline has been reported in nondiabetic dogs and has been attributed, in part, to a persistent suppressive effect on plasma free fatty acid (FFA) levels. This would be consistent with enhanced HIM2 binding to the insulin receptor in adipocytes, leading to prolongation of the hormone's antilipolytic effect. A persistent reduction in plasma FFA concentration (21) has been shown to augment insulin action in the liver and muscle. Because each subject participated in six studies, additional blood for the determination of plasma FFA concentration could not be taken. Future studies examining insulin binding in insulin target tissues is warranted from the present results.

In a previous study in type 2 diabetic patients (17), oral HIM2 produced maximal plasma insulin concentrations when given 15 min before a meal and significantly reduced the postprandial plasma glucose excursion. When given with or after the meal, absorption of HIM2 was impaired. This observation (17), combined with the present results, indicates that oral HIM2 given before meal ingestion and in appropriate doses might be a useful therapy to control postprandial hyperglycemia in type 2 diabetic patients. Despite demonstrated benefits (13,14), tight glycemic control in diabetic patients remains a largely unmet clinical challenge. Limitations of multiple daily injections include inconvenience, poor patient acceptability and adherence, and difficulty in matching postprandial insulin availability to glucose entry from the gastrointestinal tract (22). Although rapidly acting insulins are available, the inconvenience of injections persists and metabolic effects dissipate within 2 h (23), often requiring an increase in long-acting insulin dose to maintain late (2–4 h) postprandial glucose control. The development of oral, nasal, and transdermal insulin delivery preparations has limited success (22). Inhaled insulin has demonstrated good efficacy in type 1 and type 2 diabetic

patients (24,25), but currently available devices are cumbersome and the route of insulin administration is systemic.

Under the conditions of normal daily living, the gastrointestinal tract represents the route of glucose entry into the body, and insulin is secreted directly into the portal vein. High portal insulin concentrations effectively suppress hepatic glucose production (1–3), and the combination of portal hyperglycemia and portal hyperinsulinemia augment hepatic glucose uptake (2–4,26,27). Every dose of HIM2 produced a greater and more rapid (0–60 min) suppression of EGP than lispro insulin ($P < 0.01$; the 0.5- and 0.75-mg/kg HIM2 doses also produced a greater and more sustained suppression of EGP over the 240-min study period; $P < 0.01$) (Fig. 1*A* and Table 1). Another potential advantage of oral insulin administration relates to the role of the liver in first-pass insulin extraction (1). Since approximately half of the insulin secreted into the portal vein is removed by the liver, excessive peripheral hyperinsulinemia does not occur following glucose ingestion or oral insulin administration. This has clinical importance since chronic physiologic hyperinsulinemia induces moderate-to-severe peripheral (muscle) insulin resistance (6–8) and explains why intensive insulin therapy in type 2 diabetic patients has little effect to enhance peripheral tissue insulin sensitivity (28), although day-long glycemia is improved because of the suppression of hepatic glucose production.

A second aim of the present study was to compare the efficacy, time course of action, and variability of HIM2's metabolic effects with that of rapid action lispro insulin (19). HIM2 at 0.5 mg/kg produced a stimulation of total-body R_d (4.27 ± 0.35 mg · kg^{-1} · min^{-1}) similar to that observed with 0.1 units/kg lispro insulin (4.21 ± 0.41 mg · kg^{-1} · min^{-1}) over the 240-min study period (Table 1). However, the onset of action of HIM2 was faster than that of lispro. At 60 min, insulin-stimulated glucose disposal increased 2.5-fold with oral HIM2 compared with 1.5-fold with lispro ($P < 0.0$l) (Fig. 1*B*). Suppression of hepatic glucose production during the 1st hour (0–60 min) ($P < 0.0$l), as well as during the subsequent 3 h (60–240 min) ($P < 0.0$l), was also greater with a 0.5-mg/kg dose of HIM2 compared with subcutaneous lispro insulin (Fig. 1*A*). When administered to the same person on separate days, both oral HIM2 and subcutaneous lispro insulin exerted reproducible effects on R_d and EGP with coefficients of variation that were similar (Fig. 2).

In summary, we evaluated the effect of oral HIM2 on glucose kinetics in healthy subjects using the euglycemic clamp technique. Oral HIM2 suppressed EGP and stimulated tissue R_a in a dose-dependent fashion. Both effects persisted at 240 min, even though plasma HIM2 concentrations had returned to baseline. If similar actions are confirmed in type 2 diabetic patients, oral HIM2 may provide an effective and reproducible treatment for the control of postprandial glucose excursions.

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