

# Effect of Continuous Subcutaneous Insulin Infusion With Lispro on Hepatic Responsiveness to Glucagon in Type 1 Diabetes

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**OBJECTIVE** — People with type 1 diabetes frequently develop a blunted counterregulatory hormone response to hypoglycemia coupled with a decreased hepatic response to glucagon, and consequently, they have an increased risk of severe hypoglycemia. We have evaluated the effect of insulin lispro (Humalog) versus regular human insulin (Humulin R) on the hepatic glucose production (HGP) response to glucagon in type 1 diabetic patients on intensive insulin therapy with continuous subcutaneous insulin infusion (CSII).

**RESEARCH DESIGN AND METHODS** — Ten subjects on CSII were treated for 3 months with lispro and 3 months with regular insulin in a double-blind randomized crossover study. After 3 months of treatment with each insulin, hepatic sensitivity to glucagon was measured in each subject. The test consisted of a 4-h simultaneous infusion of somatostatin (450  $\mu\text{g}/\text{h}$ ) to suppress endogenous glucagon, regular insulin ( $0.15 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ), glucose at a variable rate to maintain plasma glucose near 5 mmol/l, and D-[6,6- $^2\text{H}_2$ ]glucose to measure HGP. During the last 2 h, glucagon was infused at  $1.5 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ . Eight nondiabetic people served as control subjects.

**RESULTS** — During the glucagon infusion period, free plasma insulin levels in the diabetic subjects were  $71.7 \pm 1.6$  vs.  $74.8 \pm 0.5$  pmol/l after lispro and regular insulin treatment, with plasma glucagon levels of  $88.3 \pm 1.8$  and  $83.7 \pm 1.5$  ng/l for insulin:glucagon ratios of 2.8 and 3.0, respectively (NS). However, plasma glucose increased to  $9.2 \pm 1.1$  mmol/l after lispro insulin compared with  $7.1 \pm 0.9$  mmol/l after regular insulin ( $P < 0.01$ ), and the rise in HGP was  $5.7 \pm 2.8 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  after lispro insulin versus  $3.1 \pm 2.9 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  after regular insulin treatment ( $P = 0.02$ ). In the control subjects, HGP increased by  $10.7 \pm 4.2 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  under glucagon infusion.

**CONCLUSIONS** — Insulin lispro treatment by CSII was associated with a heightened response in HGP to glucagon compared with regular human insulin. This suggests that insulin lispro increases the sensitivity of the liver to glucagon and could potentially decrease the risk of severe hypoglycemia.

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**Abbreviations:** CSII, continuous subcutaneous insulin infusion; HGP, hepatic glucose production; I:G, insulin:glucagon; IRG, immunoreactive glucagon; MW, molecular weight;  $R_a$ , rate of total glucose appearance; RIA, radioimmunoassay.

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

It is well known that people with type 1 diabetes gradually develop decreased counterregulatory hormone responses to hypoglycemia (1), with one study reporting diminished hepatic glucose production (HGP) in response to glucagon (2). Furthermore, intensified subcutaneous insulin therapy leads to further blunting of the counterregulatory hormone response to hypoglycemia compared with conventional therapy (3–5). These defects all contribute to an increased risk of severe hypoglycemia (6,7).

The impairment of glucagon secretion and action observed in type 1 diabetic patients may be due to the chronic effect of high circulating insulin levels (2,8). Pre-meal subcutaneous injections of regular human insulin in type 1 diabetic patients result in systemic hyperinsulinemia compared with normal meal-related insulin secretion in nondiabetic people (9). This nonphysiological chronic hyperinsulinemia is attributed, in part, to the delayed onset and prolonged duration of action of regular human insulin after subcutaneous injection. Insulin with a more rapid onset and shorter duration of action would decrease chronic hyperinsulinemia, simulating physiologic  $\beta$ -cell insulin secretion with meals, and may have a beneficial effect on counterregulatory hormone physiology.

Insulin lispro is an analog of native human insulin in which the natural amino acid sequence of the  $\beta$ -chain at positions 28 and 29 is inverted. These changes result in an insulin molecule with a reduced capacity for self-association (10). Insulin lispro thus exhibits a more rapid absorption and a faster pharmacodynamic action than regular human insulin after subcutaneous injection, mimicking more closely the plasma insulin dynamics of nondiabetic people in response to meals (11–14).

The objective of the present study was to evaluate the effect of insulin lispro versus regular human insulin on the HGP response to glucagon in patients with type 1 diabetes treated by continuous subcutaneous insulin infusion (CSII).

**RESEARCH DESIGN AND METHODS**

**Subjects**

Ten type 1 diabetic patients (6 men, 4 women) were submitted to a hepatic glucagon sensitivity test. Their mean age was  $36 \pm 3$  years, their mean BMI was  $25.1 \pm 1.1$  kg/m<sup>2</sup>, and the mean duration of diabetes was  $18 \pm 6$  years. None of the subjects had major microvascular or macrovascular complications. They were compared with eight normal males (mean age:  $23.8 \pm 0.6$  years; mean BMI:  $22.7 \pm 1.6$  kg/m<sup>2</sup>) serving as nondiabetic control subjects.

**Study design**

The study was a randomized double-blind crossover clinical trial designed to compare insulin lispro versus regular human insulin in type 1 diabetic patients treated by CSII. Patients who had not been on insulin pump therapy before entering the trial were hospitalized for intensive training. All of them received regular human insulin for a run-in period of 1 month if they were already on insulin pump therapy or for 3 months if they were previously on subcutaneous injections. The patients were then assigned, in random order, to either regular human insulin or insulin lispro for a 3-month period and then switched to the alternate insulin therapy (Humulin R or Humalog) for another 3 months. At the end of each 3-month treatment period, they were submitted to a hepatic glucagon sensitivity test. The effect of insulin lispro on glycemic control has already been reported as part of a larger study (15). The nondiabetic control subjects were part of a study looking at the effect of endurance training on the response of HGP to glucagon; the results from the latter study have been published recently (16). The sedentary control subjects were selected only to illustrate differences in the HGP response to glucagon between type 1 diabetic and nondiabetic subjects who were also submitted to the hepatic glucagon sensitivity test. The study was approved by the institution's ethics committee, and signed informed consent was obtained from each subject.

**Treatment**

The commercially available Disetronic H-Tron V100 pump (Biomedic, Mississauga, Ontario, Canada) was used to deliver insulin subcutaneously into the anterior abdominal wall. A premeal insulin bolus (Humalog or Humulin R) was adminis-

tered 0–5 min before each meal, based on the amount of carbohydrates. Throughout the study, capillary blood glucose was self-monitored by patients with the One-Touch II memory glucose meter (LifeScan, Burnaby, British Columbia, Canada). Patients were instructed to adjust their insulin doses to achieve fasting and premeal glucose levels between 4 and 7 mmol/l and a 1-h postprandial glucose level below 10 mmol/l.

**Measurement of hepatic glucagon sensitivity**

Subjects were studied at 7:30 A.M. after an overnight fast (~10- to 12-h postabsorptive) for the measurement of hepatic glucagon sensitivity as described previously (16). In brief, a catheter was inserted into an antecubital vein for infusion of D-[6,6-<sup>2</sup>H<sub>2</sub>]glucose, insulin, glucose, and somatostatin. A second catheter was inserted in a retrograde fashion into a hand vein of the contralateral arm, and the hand was placed in a heating box (68°C) to provide "arterialized" venous blood for sampling (17). CSII was discontinued 30 min before starting intravenous insulin infusion. During the 4-h study period, endogenous glucagon was suppressed by somatostatin infusion at 450 µg/h, insulin was replaced at  $0.15 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , and D-[6,6-<sup>2</sup>H<sub>2</sub>]glucose was administered as a prime-constant infusion (250 mg at 2.5 mg/min) to measure HGP. The first 2 h served as an equilibration period for the tracer, while plasma glucose was clamped at 5 mmol/l by variable glucose infusion. Over the last 2 h, glucagon was infused at  $1.5 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , and glucose infusion was then maintained constant at the rate achieved by 120 min. Blood samples were drawn every 10 or 15 min for the determination of unlabeled and labeled plasma glucose, free plasma insulin, and plasma glucagon.

**Analytical methods**

Plasma glucose was measured by the hexokinase method with the COBAS BIO Analyzer (Roche Analytical Instruments, Nutley, NJ). The D-[6,6-<sup>2</sup>H<sub>2</sub>]glucose was purchased from Isotec (Miamisburg, OH) with a purity of 99%. Plasma glucose isotopic enrichment was assessed by combined gas chromatography–mass spectrometry (model 5890–5970; Hewlett-Packard, Palo Alto, CA) after derivatization according to Küry and Keller (18). Plasma glucagon was measured by radioimmunoassay (RIA) after precipitation by polyethylene glycol 6000

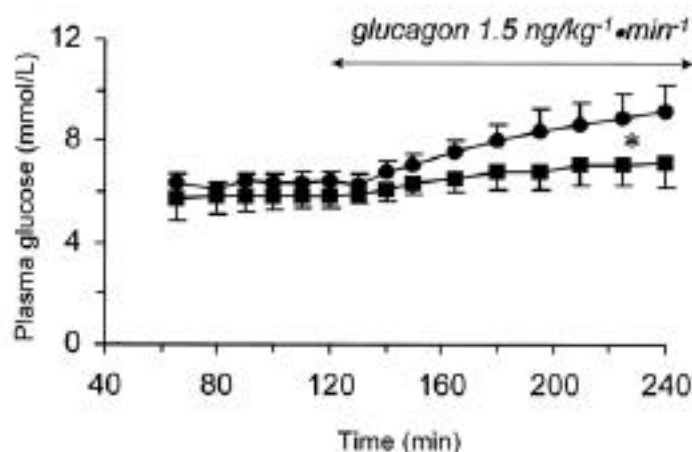
(Eastman-Kodak, Rochester, NY) (19,20). This eliminates big plasma glucagon and the 9,000 molecular weight (MW) immunoreactive glucagon (IRG), which together account for 80% of total IRG in the basal state in normal subjects (21). It does not eliminate the 2,000 MW IRG, which constitutes <4% of total basal IRG. Recovery of added glucagon 3,500 MW to glucagon-free plasma is >90% (20). Using this methodology, the coefficient of variation for the glucagon assay was  $6.7 \pm 1.4\%$ . Free insulin was measured by RIA (22,23). HbA<sub>1c</sub> was assayed by fast protein liquid chromatography using mono-S HR 5/5 columns (Pharmacia, Dorval, Quebec, Canada) (normal = 3.5–5.7%).

**Statistical analysis**

Only 8 of the 10 diabetic patients were analyzed because of technical problems in 2 subjects (malfunction of the glucagon infusion pump in one case and an overdosage error of glucagon infusion in the other). The rates of total glucose appearance ( $R_a$ ) were determined from D<sub>2</sub>-glucose enrichment. Values were calculated according to the non-steady-state equations of Steele (24), using 200 ml/kg as the glucose distribution volume and 0.65 for the pool fraction. HGP was calculated by subtracting the glucose infusion rate from  $R_a$ .

All values are expressed as means  $\pm$  SEM. Because all diabetic subjects served as their own control, comparisons between the two treatments were made by paired Student's *t* test. Comparisons between the diabetic and nondiabetic subjects were made by unpaired Student's *t* test. *P* values <0.05 are reported as significant.

**RESULTS** — The data on insulin requirement, HbA<sub>1c</sub>, and capillary blood glucose profile have already been published elsewhere as part of a group of 30 type 1 diabetic patients (15). While there was no change in insulin requirement, lispro treatment resulted in a better HbA<sub>1c</sub> ( $7.66 \pm 0.13$  vs.  $8.0 \pm 0.16\%$ ; *P* = 0.04), and a significant decrease in hypoglycemia was observed during the lispro ( $12.7 \pm 1.6$  to  $8.6 \pm 1.4$  events per 30 days; *P* = 0.035) but not during the regular insulin treatment ( $12.7 \pm 1.6$  to  $10.8 \pm 1.8$  events per 30 days; NS). In the subset of patients who underwent hepatic glucagon sensitivity measurement, HbA<sub>1c</sub> was  $6.6 \pm 0.2\%$ , and the number of documented hypoglycemic events (<4 mmol/l) was  $16 \pm 3$  per patient per month before randomization; these parameters were not significantly



**Figure 1**—Plasma glucose concentration in response to glucagon infusion after 3 months of treatment with insulin lispro (●) and regular human insulin (■) using CSII. All values are expressed as means  $\pm$  SEM. \* $P < 0.01$ .

affected by 3 months of intensive treatment with insulin lispro or regular human insulin by CSII.

#### Hepatic glucagon sensitivity

In the diabetic subjects, during the glucagon infusion period, mean free plasma insulin levels ( $71.7 \pm 1.6$  and  $74.75 \pm 0.5$  pmol/l) and plasma glucagon concentrations ( $88.3 \pm 1.8$  and  $83.7 \pm 1.5$  ng/l) were similar after insulin lispro and regular human insulin treatment. The insulin:glucagon (I:G) ratio was  $2.8 \pm 0.07$  vs.  $3.0 \pm 0.07$  after insulin lispro and regular human insulin treatment (NS). In the control subjects, plasma insulin ( $96.2 \pm 1.2$  pmol/l) and glucagon ( $145.3 \pm 2.1$  ng/l) were slightly higher, but the I:G ratio was similar ( $2.7 \pm 0.08$ ).

In the diabetic group, the mean plasma glucose level during the last 30 min of the equilibration period (90–120 min) was stable at  $6.1 \pm 0.1$  mmol/l after both treatments. During the glucagon infusion period, plasma glucose increased but more so after insulin lispro than after regular human insulin treatment ( $9.2 \pm 1.1$  vs.  $7.1 \pm 0.3$  mmol/l;  $P < 0.01$ ) (Fig. 1). In the control subjects, mean plasma glucose was  $5.7 \pm 0.4$  mmol/l during the last 30 min of the equilibration period and increased to  $8.9 \pm 0.8$  mmol/l during glucagon infusion.

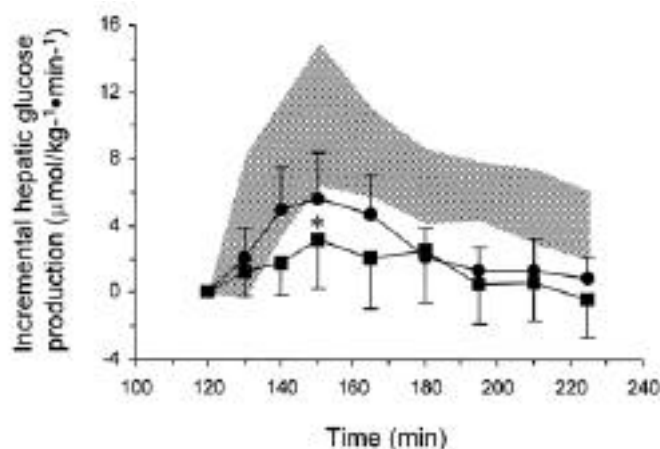
In the diabetic subjects, during the last 30 min of the equilibration period, the plasma isotopic enrichment was identical and very stable after insulin lispro and regular insulin treatment ( $0.89 \pm 0.08$  vs.  $0.89 \pm 0.06$ ). During this period, HGP was  $8.2 \pm 0.3$  and  $6.7 \pm 0.6$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  (NS) after insulin lispro and regular insulin treat-

ment, respectively. When glucagon was infused at  $1.5 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , isotopic enrichment decreased after both insulin lispro and regular insulin treatment, but more so after the former ( $0.79 \pm 0.06$  vs.  $0.85 \pm 0.07$ ), and then it gradually increased to reach  $0.86 \pm 0.06$  and  $0.90 \pm 0.09$ , respectively, by the end of the study. During this period, HGP increased to a maximum of  $13.8 \pm 3.0$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  after insulin lispro treatment compared with  $9.8 \pm 2.7$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  after regular human insulin treatment at 150 min ( $P < 0.01$ ). HGP then decreased gradually to  $9.0 \pm 2.0$  and  $6.3 \pm 2.0$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , respectively, by the end of the study (NS). The peak increase in HGP above the rate achieved by the end of the equilibration period was  $5.7 \pm 2.8$   $\mu\text{mol} \cdot$

$\text{kg}^{-1} \cdot \text{min}^{-1}$  after insulin lispro treatment compared with  $3.1 \pm 2.9$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  after regular human insulin treatment (Fig. 2) ( $P = 0.02$ ). In the nondiabetic control subjects, the mean increase in HGP in response to glucagon infusion was  $10.7 \pm 4.2$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  by 150 min (Fig. 2). This was significantly different from regular human insulin treatment ( $P < 0.01$ ), but not from insulin lispro treatment.

**CONCLUSIONS** — The present study was designed to evaluate the effect of insulin lispro versus regular human insulin treatment on HGP in response to glucagon in patients with type 1 diabetes on intensive insulin therapy using CSII. We confirmed the observation of Orskov et al. (2) that type 1 diabetic patients have hepatic resistance to glucagon. Furthermore, we show that insulin lispro treatment can partially restore hepatic sensitivity to glucagon. The HGP response to glucagon still remained blunted compared with nondiabetic people (Fig. 2) (16). In a subset of patients ( $n = 10$ ), we have also shown that 3 months of CSII using insulin lispro did not affect counterregulatory hormones to hypoglycemia despite better glycemic control compared with regular human insulin by CSII (25). It is possible, however, that longer treatment with insulin lispro could have totally normalized the observed hepatic defect and improved the counterregulatory responses to hypoglycemia.

How can insulin lispro improve the HGP response to glucagon? Several possible explanations can be offered, including decreased chronic hyperinsulinemia and/or improved hepatic glycogen stores.



**Figure 2**—Increased HGP in response to glucagon after 3 months of treatment with insulin (●) or regular human insulin (■) using CSII compared with nondiabetic control subjects (shaded area). All values are expressed as means  $\pm$  SEM \* $P < 0.02$ .

It is well known that insulin regulates glucagon synthesis and secretion (26) by inhibiting glucagon gene transcription (27,28). Intensive insulin therapy has been shown to induce hyperinsulinemia (9), resulting in downregulation of insulin receptors (29–32). Insulin resistance releases the tonic inhibition on glucagon synthesis causing hyperglucagonemia (33–35). There are a number of experimental observations indicating that high glucagon levels downregulate glucagon's own receptors (36–39). At least in animal studies, this leads to decreased hepatic glycogenolysis in response to glucagon (36,38,40). We propose that hepatic glucagon resistance in type 1 diabetic patients is due mainly to slowly absorbed subcutaneous insulin, resulting in chronic hyperinsulinemia, insulin resistance, hyperglucagonemia, and decreased glucagon receptors. In fact, when plasma glucose and insulin are normalized by intravenous insulin infusion using artificial pancreas (Biostator), plasma glucagon can also be normalized (41). It is therefore possible that in our study the faster-absorbing insulin lispro produced a more physiological insulin profile, particularly after meals, resulting in lower overall insulin levels, reduced insulin resistance, decreased glucagon levels, and an improved hepatic response to glucagon. The lower overall circulating insulin levels during insulin lispro treatment is supported by the study of Jehle et al. (42), who have shown that intensified insulin therapy with lispro increased the number and the affinity of insulin receptors on circulating monocytes to a level similar to that observed in healthy subjects. They concluded that the improved insulin receptor status during lispro treatment was caused by its more physiological pharmacokinetic profile.

A number of studies have shown that the initial increase in HGP in response to glucagon is due to glycogen breakdown (43,44). It has been suggested that poorly controlled type 1 diabetic patients have a defect in net hepatic glycogen synthesis leading to decreased liver glycogen reserves (45). It is likely that under these conditions, the lower postprandial I:G ratio plays an important role in this defect. Insulin lispro gives an earlier and higher insulin peak after subcutaneous injection compared with regular human insulin, mimicking more closely the postprandial plasma insulin rise observed in nondiabetic people (11,13,15). This improvement of the postprandial plasma insulin profile could theoretically improve hepatic glycogen synthesis; this is supported

by animal studies where treatment with a fast-acting insulin analog increased glycogen deposition in the liver and skeletal muscles (46). It is therefore possible that the augmented HGP in response to glucagon after insulin lispro treatment is due, at least in part, to higher liver glycogen content.

In conclusion, our data on type 1 diabetic patients under intensive insulin therapy by CSII indicate that insulin lispro is associated with heightened hepatic glucagon sensitivity. We propose that this is due to an improved plasma insulin profile and decreased chronic hyperinsulinemia. It is suggested that this beneficial effect resulting from insulin lispro treatment could decrease the risk of severe hypoglycemia.

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