Glucagon-like Peptide-1 (7-37) Augments Insulin-Mediated Glucose Uptake in Elderly Patients With Diabetes

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Background. Glucagon-like peptide-1 (GLP-1) is an intestinal insulinotropic hormone that augments glucose-induced insulin secretion in patients with type 2 diabetes. It has also been proposed that a substantial component of the glucose-lowering effects of GLP-1 occurs because this hormone enhances insulin-mediated glucose disposal. However, interpretations of the studies have been controversial. This study determines the effect of GLP-1 on insulin-mediated glucose disposal in elderly patients with type 2 diabetes.

Methods. Studies were conducted on 8 elderly patients with type 2 diabetes (age range, 76 ± 1 years; body mass index, 28 ± 1 kg/m²). Each subject underwent two 180-minute euglycemic (insulin infusion rate, 40 mU/m²/min) insulin clamps in random order. Glucose production (Ra) and disposal (Rd) rates were measured using tritiated glucose methodology. In one study, glucose and insulin alone were infused. In the other study, a primed-continuous infusion of GLP-1 was administered at a final rate of 1.5 pmol · kg⁻¹ · min⁻¹ from 30 to 180 minutes.

Results. Glucose values were similar between the control and GLP-1 infusion studies. 120- to 180-minute insulin values appeared to be higher during the GLP-1 infusion study (control, 795 ± 63 pmol/l; GLP-1, 1140 ± 275 pmol/l; p = not significant [NS]). The higher insulin values were largely due to 2 subjects who had substantial insulin responses to GLP-1 despite euglycemia and hyperinsulinemia. The 120- to 180-minute insulin values were similar in the other 6 subjects (control, 746 ± 35 pmol/l; GLP-1, 781 ± 41 pmol/l; p = NS). Basal (control, 2.08 ± 0.05 mg/kg/min; GLP-1, 2.13 ± 0.04 mg/kg/min; p = NS) and 120- to 180-minute (control, 0.50 ± 0.18 mg/kg/min; GLP-1, 0.45 ± 0.14 mg/kg/min; p = NS) Ra was similar between studies. The 120- to 180-minute Rd values were higher during the GLP-1 infusion studies (control, 4.73 ± 0.39 mg/kg/min; GLP-1, 5.52 ± 0.43 mg/kg/min; p < .01). When the 2 subjects who had significant insulin responses to GLP-1 during the euglycemic clamp were excluded, the 120- to 180-minute Rd values were still higher in the GLP-1 infusion study (control, 5.22 ± 0.32 mg/kg/min; GLP-1, 6.05 ± 0.37 mg/kg/min; p < .05).

Conclusions. We conclude that GLP-1 may enhance insulin sensitivity in elderly patients with diabetes.

GLUCAGON-like peptide-1 (GLP-1) is a gastrointestinal hormone secreted from the intestine in response to food (1). GLP-1 is a potent stimulator of glucose-induced insulin release (1,2). GLP-1 augments insulin release in middle-aged patients with type 2 diabetes (3,4) and is therefore a potentially promising agent for the treatment of diabetes. Because in vitro studies have found that GLP-1 augments insulin-mediated glucose uptake (5–11), it has been proposed that GLP-1 might also have insulin-augmenting properties in peripheral tissues. However, the results of in vivo studies on the effect of GLP-1 on insulin-mediated glucose disposal in normal and diabetic subjects are conflicting (12–18).

Aging is characterized by a progressive increase in the prevalence of diabetes (19,20). The results of studies of therapeutic interventions in younger patient populations cannot automatically be extrapolated to elderly individuals, and these interventions must be specifically evaluated in an elderly population. One metabolic abnormality in obese elderly patients with diabetes is resistance to insulin-mediated glucose disposal (21). If GLP-1 enhances insulin-mediated glucose disposal, it would be an ideal treatment for elderly patients with diabetes. We undertook the following experiments to determine if GLP-1 has insulin-augmenting activity in elderly patients with diabetes.

Methods
These studies were conducted on 8 elderly patients with diabetes (age range, 76 ± 1 years; body mass index, 28 ± 1 kg/m²). Patients were recruited from the Vancouver Hospital Diabetes Center. The mean duration of diabetes was 6 ± 1 years, mean HbA₁c (hemoglobin A) was 7.4% ± 0.3%, and mean arterial blood pressure was 93 ± 6 mm Hg. All sub-
jects were free of clinically apparent microvascular, macrovascular, or neuropathic complications. Patients with hypertension were not excluded. Four of the subjects were treated with sulfonlurea drugs, five were treated with metformin, and five were being treated with angiotensin-converting enzyme, or ACE, inhibitors for hypertension. All medications were stopped 2 weeks prior to each study. This study was approved by the Committee on Human Investigation at the University of British Columbia. All subjects gave written informed consent prior to participation.

All subjects were asked to consume a weight-maintaining diet without carbohydrate restriction and to maintain their usual level of physical activity for 3 days prior to testing. Subjects were studied in our clinical research center after a 12-hour overnight fast. All subjects arrived in the clinical research center by 6:30 AM, and all tests were begun by 7:30 AM.

Each subject underwent two euglycemic clamp studies in random order, separated by at least 1 month (22). In each study, 18 gauge catheters were inserted into an antecubital vein for infusion of glucose and tracer and into a contralateral hand vein for sampling of “arterialized” venous blood (23). At the −120-minute point, a priming dose of 424 ± 38 nCi/kg of sterile and pyrogen-free 3-[^3]H]-glucose (New England Nuclear, Boston, MA) was administered at −120 minutes, followed by a constant intravenous infusion of 2.76 ± 0.11 nCi/kg/min for the duration of the experiment. From the −20- to 0-minute points, three blood samples were taken at 10-minute intervals to measure basal glucose, insulin, C-peptide, GLP-1, glucagon, and glucose-specific activity. At time 0, an insulin infusion (Humulin R, Eli Lilly, Indianapolis, IN) was started at 40 mU/m².min. Blood glucose was allowed to fall from fasting levels to 5.5 mmol/l. Once glucose was at 5.5 mmol/l, a glucose infusion was begun and the blood glucose was kept at that level for the duration of the study. The glucose solution was “spiked” with tritiated glucose in order to maintain a constant glucose-specific activity as previously described (21). This “hot Gin” method has been shown to minimize the errors associated with the use of non-steady-state equations to calculate glucose turnover. This method resulted in stable specific activity values in both the control (basal, 1.5 ± 0.1 nCi/mg; 120–180 minutes, 1.4 ± 0.1 nCi/mg) and the GLP-1 studies (basal, 1.4 ± 0.1 nCi/mg; 120–180 minutes, 1.4 ± 0.1 nCi/mg). Each euglycemic clamp duration was 180 minutes. In one study, insulin and glucose were infused; in the other study, GLP-1 was infused at a final rate of 1.5 pmol/kg/min, from 30 to 180 minutes, as previously described, along with insulin and glucose (16).

Blood samples were taken every 5 minutes throughout the study for a measurement of glucose and every 15 minutes to measure insulin, C-peptide, GLP-1, glucagon, and glucose-specific activity.

GLP-1 (7-37) was synthesized in the Massachusetts General Hospital Biopolymer Core Facility (16). This preparation is more than 99% pure and displays a single peak on high-performance liquid chromatography. The peptide is filtered through 0.2-μM nitrocellulose filters (Millipore, Bedford, MA) before it is lyophilized in vials under sterile conditions for single volunteer use. The samples were analyzed and shown to be both sterile and pyrogen free; the net peptide content was used for dose calculations.

### Analytical Technique

Blood samples were collected in heparinized syringes. Plasma glucose was measured immediately at the bedside using a YSI Glucose Analyzer (Yellow Springs Instruments, Yellow Springs, OH). The remaining blood was placed in prechilled test tubes containing diprotin A (for measurement of GLP-1), aprotonin (400 KIU/ml), and EDTA (1.5 mg/ml; for measurement of C-peptide, glucagon, and insulin), and centrifuged at 4°C. The samples were stored in a −70°C freezer until analysis. Insulin, glucagon, C-peptide, and total and active GLP-1 were measured by radioimmunoassays as previously described (24).

### Statistical Analyses

The rates of total glucose production (Ra) and disposal (Rd) were calculated according to the non-steady-state equations of Steele as modified for the use of “hot Gin” (21). The volume of distribution of glucose was assumed to be 210 ml/kg. Endogenous glucose production was estimated as the difference between the calculated total appearance rate and the exogenous glucose infusion for the appropriate time interval during the clamp.

Differences between studies were evaluated with the two-tailed paired t tests, except when the data were not normally distributed, when the Wilcoxon signed rank test was used. Except where otherwise stated, results are presented as mean ± SEM. A p value < .05 was considered significant in all analyses.

### Results

Fasting glucose and hormone values are shown in Table 1 and did not differ between studies. Plasma glucose, insulin, and C-peptide levels for the studies are illustrated in Figure 1. The 120- to 180-minute control (5.4 ± 0.1 mmol/l; GLP-1, 5.4 ± 0.1 mmol/l; p = not significant [NS]) glucose values were similar between studies. The 120- to 180-minute insulin values were higher during the GLP-1 infusion study (control, 795 ± 63 pmol/l; GLP-1, 1140 ± 275 pmol/l; p < .06, Wilcoxon signed rank test). The higher insulin values were largely due to 2 subjects who had substantial insulin responses to GLP-1 (Figure 2). The 120- to 180-minute insulin values were not significantly increased in the other 6 subjects (control, 746 ± 35 pmol/l; GLP-1, 781 ± 41 pmol/l; p = NS; Figure 2). Basal C-peptide levels were similar in the two studies (Table 1). The 120- to 180-minute plasma C-peptide values appeared to be higher during the GLP-1 infusion study (control, 0.4 ± 0.1 nmol/l; GLP-1, 1.8 ± 0.7 nmol/l; p < .06, Wilcoxon signed rank test). Once again, the values were skewed by 2 subjects who had substantial

### Table 1. Fasting Glucose and Hormone Values

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>GLP-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mmol/l</td>
<td>6.8 ± 0.5</td>
<td>6.8 ± 0.4</td>
</tr>
<tr>
<td>Insulin, pmol/l</td>
<td>131 ± 29</td>
<td>137 ± 27</td>
</tr>
<tr>
<td>C-peptide, nmol/l</td>
<td>0.8 ± 0.2</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>Glucagon, pmol/l</td>
<td>21 ± 2</td>
<td>24 ± 3</td>
</tr>
<tr>
<td>Total GLP-1, pmol/l</td>
<td>6 ± 2</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>Active GLP-1, pmol/l</td>
<td>2 ± 1</td>
<td>3 ± 2</td>
</tr>
</tbody>
</table>

*Note: GLP-1 = glucagon-like peptide-1.*
insulin responses to GLP-1 (Figure 2). C-peptide values were not significantly increased in the other 6 subjects (control, 0.5 ± 0.1 nmol/l; GLP-1, 1.0 ± 0.2 nmol/l; p = NS; Figure 2).

Plasma glucagon and total and active GLP-1 levels for the euglycemic clamp studies are illustrated in Figure 3. The 120- to 180-minute total (control, 4 ± 1 pmol/l; GLP-1, 19 ± 11 pmol/l; p < .0001) and active (control, 3 ± 2 pmol/l; GLP-1, 19 ± 2 pmol/l; p < .0001) GLP-1 values were substantially higher during the GLP-1 study, whereas glucagon (control, 16 ± 2 pmol/l; GLP-1, 16 ± 2 pmol/l; p = NS) values were similar.

Ra and Rd during the euglycemic clamps are shown in Figure 4. Basal Ra/Rd was similar between studies (control, 2.08 ± 0.05 mg/kg/min; GLP-1, 2.13 ± 0.04 mg/kg/min; p = NS). The 120- to 180-minute Ra values were similar in each study (control, 0.50 ± 0.18 mg/kg/min; GLP-1, 0.45 ± 0.14 mg/kg/min; p = NS). The 120- to 180-minute Rd values were higher during the GLP-1 infusion studies (control, 4.73 ± 0.39 mg/kg/min; GLP-1, 5.52 ± 0.43 mg/kg/min; p < .01), an increase of 15% ± 3%. When the 2 subjects who had significant insulin responses to GLP-1 during the euglycemic clamp were excluded, the 120- to 180-minute Rd values were still higher in the GLP-1 infusion study (control, 5.22 ± 0.32 mg/kg/min; GLP-1, 6.05 ± 0.37 mg/kg/min; p < .05).

DISCUSSION

The majority of in vitro studies have found that GLP-1 enhances insulin-mediated glucose disposal in a variety of tissues (5–11). However, the data from in vivo studies are conflicting. GLP-1 has been reported to enhance or have no effect on insulin-mediated glucose disposal in healthy volunteers and patients with type 1 diabetes (12–16). Two studies found no effect of GLP-1 on insulin-mediated glucose uptake in middle-aged patients with type 2 diabetes (17, 18).

In the last few years, we have systematically evaluated the metabolic profile of elderly patients with diabetes. One of the metabolic defects in obese elderly patients is resistance to insulin-mediated glucose disposal (21). The purpose of this study was to specifically evaluate the effect of GLP-1 on insulin-mediated glucose disposal in these patients. Our data suggest that GLP-1 may modestly increase insulin-mediated glucose disposal.

It is important to compare our findings with previous studies in middle-aged subjects. Ahrén and colleagues (18) administered GLP-1 at a similar infusion rate to middle-aged patients with diabetes during a 2-hour euglycemic clamp. Similar to our study, they found that insulin levels were higher during the GLP-1 infusion, but they demonstrated no change in insulin sensitivity. They do not report whether the increase in insulin levels occurred in a subset of subjects. The discrepancy between the reports may be related to the duration of the GLP-1 infusion. The Rd difference between the GLP-1 and control study continued to increase throughout our study, and Ahrén and colleagues might have detected a significant difference in insulin sensitivity if they had infused GLP-1 for a longer period of time. Vella and colleagues (17) administered glucose and insulin in a manner typical of that occurring during a meal. GLP-1 was infused along with glucose and insulin. These investi-
MENEILLY ET AL.

gators found no effect of GLP-1 on glucose disposal or hepatic glucose production. Once again, the discrepancy between studies may relate to experimental design. In Vella and colleagues’ study, insulin levels were substantially lower and glucose levels were substantially higher than in our studies, and it is possible that GLP-1 may have different effects on glucose disposal at different levels of glucose and insulin. In addition to differences in the age of the subjects studied and the experimental design, one of the major differences between our study and those described previously is the homogeneity of the subjects. Heterogeneity among subjects can blur the results of small physiological studies in patients with type 2 diabetes. Our patients were very similar with regard to age, duration of diabetes, body mass, and glycemic control, and this may explain in part the differing results. Nevertheless, there are discrepant results between studies that have attempted to address the effects of GLP-1 on glucose disposal in patients with diabetes. To resolve this issue completely, studies will need to be conducted in C-peptide-negative patients with diabetes who are carefully matched with regard to duration of diabetes, metabolic control, body composition, etc.

Our study had several other important findings. As reported previously, total GLP-1 levels were higher than active GLP-1 levels, probably due to metabolism by dipeptidyl peptidase IV (24,25). In the control study, endogenous insulin secretion, as measured by C-peptide levels, was completely suppressed by hyperinsulinemia. A new finding of our study is that GLP-1 can overcome the effects of hyperinsulinemia on endogenous insulin secretion in some patients at euglycemia. In the 6 subjects who did not experience this breakthrough effect, it is possible that the increased insulin secretion that occurred in response to GLP-1 may have contributed to the increase in glucose disposal. However, it should be noted that the increase in insulin levels in these subjects contributed very little to overall insulin levels during the clamp. GLP-1 inhibits endogenous glucagon secretion in middle-aged patients with diabetes (26). We demonstrated that GLP-1 does not add to the effect of insulin on glucagon secretion in elderly patients with diabetes.

In conclusion, GLP-1 may enhance insulin-mediated glucose disposal in elderly persons with diabetes. The results of this study, coupled with the results of other studies demonstrating the substantial insulinotropic effects of this peptide, will be the basis for clinical trials designed to evaluate the effectiveness of GLP-1 in elderly patients with diabetes.

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