Effect of Glucagon-Like Peptide 1 on Non–Insulin-Mediated Glucose Uptake in the Elderly Patient With Diabetes

GRAYDON S. MENEILLY, MD[1]
CHRISTOPHER H.S. MCINTOSH, PHD[2]
RAYMOND A. PEDERSON, PHD[2]
JOEL F. HABENER, MD[6,7]
RONALD GINGERICH, PHD[5]
JOSEPHINE M. EGAN, MD[4]
DIANE T. FINEGOOD, PHD[3]
DARIUSH ELAHI, PHD[4,6]

An important cause of elevated glucose levels in elderly patients with diabetes is an alteration in non–insulin-mediated glucose uptake (NIMGU). Glucagon-like peptide 1 (GLP-1) is an intestinal insulinotropic hormone. It has been proposed that this hormone also lowers glucose levels by enhancing NIMGU. This study was conducted to determine whether GLP-1 augments NIMGU in elderly patients with diabetes, a group in which NIMGU is known to be impaired. Studies were conducted on 10 elderly patients with type 2 diabetes (aged 75 ± 2 years, BMI 27 ± 1 kg/m^2) who underwent paired 240-min glucose clamp studies. In each study, octreotide was infused to suppress endogenous insulin release, and tritiated glucose methodology was used to measure glucose production and disposal rates. For the first 180 min, no glucose was infused. From 180 to 240 min, glucose was increased to 11 mmol/l using the glucose clamp protocol. In the GLP-1 study, GLP-1 was infused from 30 to 240 min. In a subsequent control study, insulin was infused using the glucose clamp protocol from 30 to 240 min to match the insulin levels that occurred during the GLP-1 infusion study. During hyperglycemia, GLP-1 enhanced glucose disposal (control study: 2.52 ± 0.19 mg · kg^{-1} · min^{-1}; GLP-1 study: 2.90 ± 0.17 mg · kg^{-1} · min^{-1}; $P < 0.0001$). Hepatic glucose output was not different between studies. We conclude that GLP-1 may partially reverse the defect in NIMGU that occurs in elderly patients with diabetes.

Diabetes Care 24:1951–1956, 2001

Glucose disposal in humans occurs as a result of both insulin-mediated glucose uptake (IMGU) and non–insulin-mediated glucose uptake (NIMGU). In normal subjects, ~75% of glucose disposal (R_d) under euglycemic conditions occurs as a result of NIMGU, primarily in the central nervous system and, to a lesser extent, in other tissues such as the splanchnic bed, blood cells, the peripheral nerves, and skeletal muscle (1–6). Under hyperglycemic conditions, the proportion of NIMGU occurring in skeletal muscle increases substantially (1,7), and the quantitative importance of NIMGU to overall glucose disposal is similar to the quantitative importance of IMGU (8). In insulin-resistant conditions such as diabetes, ~80% of glucose uptake after a meal occurs as a result of NIMGU (8).

NIMGU is impaired in healthy older individuals at fasting levels but functions normally during hyperglycemia (2,9,10). In elderly patients with type 2 diabetes (11), there is a marked impairment in NIMGU during both euglycemia and hyperglycemia. Because NIMGU constitutes such a large proportion of glucose uptake in patients with diabetes, enhancement of NIMGU could have a profound effect on glycemic control in these patients.

Glucagon-like peptide 1 (GLP-1) is a gastrointestinal hormone secreted from the intestine in response to food (12,13). It has been suggested that GLP-1 may enhance NIMGU, but studies in normal subjects and middle-aged patients with type 2 diabetes have produced conflicting results (14–18).

We conducted the following studies with the hypothesis that GLP-1 would reverse the defect in NIMGU in elderly patients with diabetes.

RESEARCH DESIGN AND METHODS

— Elderly patients with type 2 diabetes were recruited for the study from the Diabetes Center at the Vancouver Hospital ($n = 10$, age 75 ± 2 years, BMI 27 ± 1 kg/m^2). Patients were excluded if they showed evidence of clinically significant complications from their diabetes. Five patients were being treated for hypertension with ACE inhibitors. Seven patients were being treated with metformin, and three with sulfonylureas. All medications were discontinued 72 h before each study. No patient was being treated with insulin. The mean HbA_{1c} was 7.1 ± 0.2%. The study was approved by the University of British Columbia Committee on Human Investigation. All subjects gave written informed consent before participating.

Subjects consumed a diet containing at least 200 g carbohydrates for 3 days before each test. Testing began at 0700 after a 12-h overnight fast. Each subject underwent two glucose clamp studies ac-

From the Departments of [1]Medicine and [2]Physiology, University of British Columbia, Vancouver, Canada; the [3]School of Kinesiology, Simon Fraser University, Burnaby, Canada; the [4]Laboratory of Clinical Physiology, Gerontology Research Center, National Institute on Aging, National Institutes of Health, Baltimore, Maryland; [5]Linco Research, St. Charles, Missouri; the [6]Department of Medicine, Massachusetts General Hospital, Boston, Massachusetts; and the [7]Laboratory of Molecular Endocrinology, Howard Hughes Medical Institute, Harvard Medical School, Boston, Massachusetts.

Address correspondence and reprint requests to Dr. G.S. Meneilly, Vancouver Hospital and Health Sciences Centre, UBC Site, 2211 Wesbrook Mall, Rm. S 169, Vancouver, BC V6T 2B5, Canada. E-mail: meneilly@interchange.ubc.ca.

Received for publication 27 February 2001 and accepted in revised form 4 June 2001.

Abbreviations: ANOVA, analysis of variance; DPIV, dipeptidyl peptidase IV; GLP-1, glucagon-like peptide 1; IMGU, insulin-mediated glucose uptake; NIMGU, non–insulin-mediated glucose uptake; R_a, rate of glucose production; R_d, rate of glucose disposal.

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

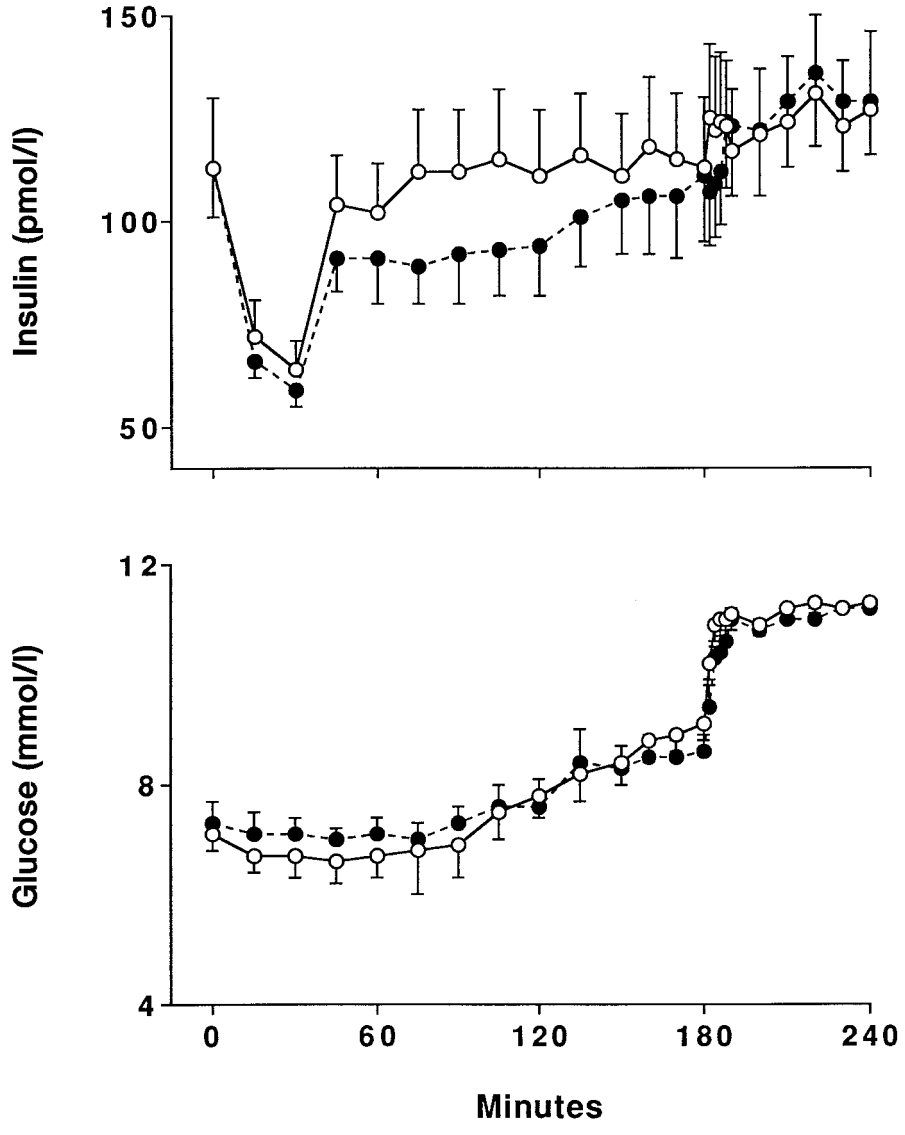

Figure 1—*Glucose and insulin values during the glucose clamp studies. ○, Control study; ●, GLP-1 study.*

cording to the method of Andres et al. (19). In all studies, intravenous lines were inserted into an antecubital vein for an infusion of glucose and into a contralateral hand vein for sampling of "arterialized" venous blood (20). Glucose production (R_a) and R_d rates were determined by a primed-constant infusion of tritiated glucose (DuPont-NEN, Boston, MA). All subjects received a priming dose at −120 min, followed by a constant infusion to 240 min. The priming dose in the patients with diabetes was adjusted based on the fasting glucose level as previously described (21). At −20 min, three blood samples were taken to measure basal glucose, insulin, glucagon, GLP-1, and glucose specific activity. At time 0, an infusion of octreotide (Sandostatin; Sandoz, Basel) was commenced at a rate of 30 ng · kg^{-1} · min^{-1} and continued for 240 min. This octreotide infusion protocol has been previously shown to adequately suppress endogenous insulin release during glucose infusion (22). No glucose was infused for the first 180 min. At 180 min, glucose was raised to 11 mmol/l using the glucose clamp protocol. Glucose was kept at that level until 240 min. In the first study, GLP-1 was infused in a primed continuous manner from 30 to 240 min at a rate of 1.5 pmol/l · kg^{-1} · min^{-1}. In the second study, human insulin (Humulin R; Eli Lilly, Indianapolis, IN) was infused from 30 to 240 min using the glucose clamp protocol in order to achieve insulin levels that were comparable to the GLP-1 infusion study. Blood samples were taken every 5 min throughout the study to measure glucose and at regular intervals to measure insulin, glucagon, GLP-1, and glucose specific activity. The coefficient of variation of plasma glucose during the hyperglycemic part of the study did not exceed 5% in any subject.

GLP-1(7–37) was synthesized in the MGH Biopolymer Core Facility (23). This preparation is >99% pure and displays a single peak on high-performance liquid chromatography (HPLC). The peptide was filtered through 0.2 μmol/l vitrocellulose filters (Millipore, Bedford, MA) before it was lyophilized in vials under sterile conditions for single use. Samples were analyzed and shown to be both sterile and pyrogen free; net peptide content was used for dose calculations.

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 glucagon and insulin) and centrifuged at 4°C. Samples were stored in a −70°C freezer until analysis. Insulin, glucagon, and total GLP-1 were measured by radioimmunoassays as previously described (23). Total GLP-1 antibody is directed at the COOH-terminal end of the molecule and thus recognizes both the intact and truncated forms of GLP-1. Active GLP-1 was measured in the assay services department at Linco Research using an enzyme-linked immunosorbent assay technique. This assay measures both the 7–36 and the 7–37 GLP-1 moieties and completely excludes the 9–36 and 9–37 truncated forms.

R_a and R_d were calculated using Steele's equations for nonsteady-state conditions (11). The volume of distribution of glucose was 210 ml/kg. Peripheral glucose effectiveness (*Sg* uptake) was calculated for each individual by the following formula (11):

$$S_g \text{ uptake} = \frac{R_d\,(210\text{–}240\text{ min}) - R_d\,(150\text{–}180\text{ min})}{\text{Glucose}\,(210\text{–}240\text{ min}) - \text{Glucose}\,(150\text{–}180\text{ min})}$$

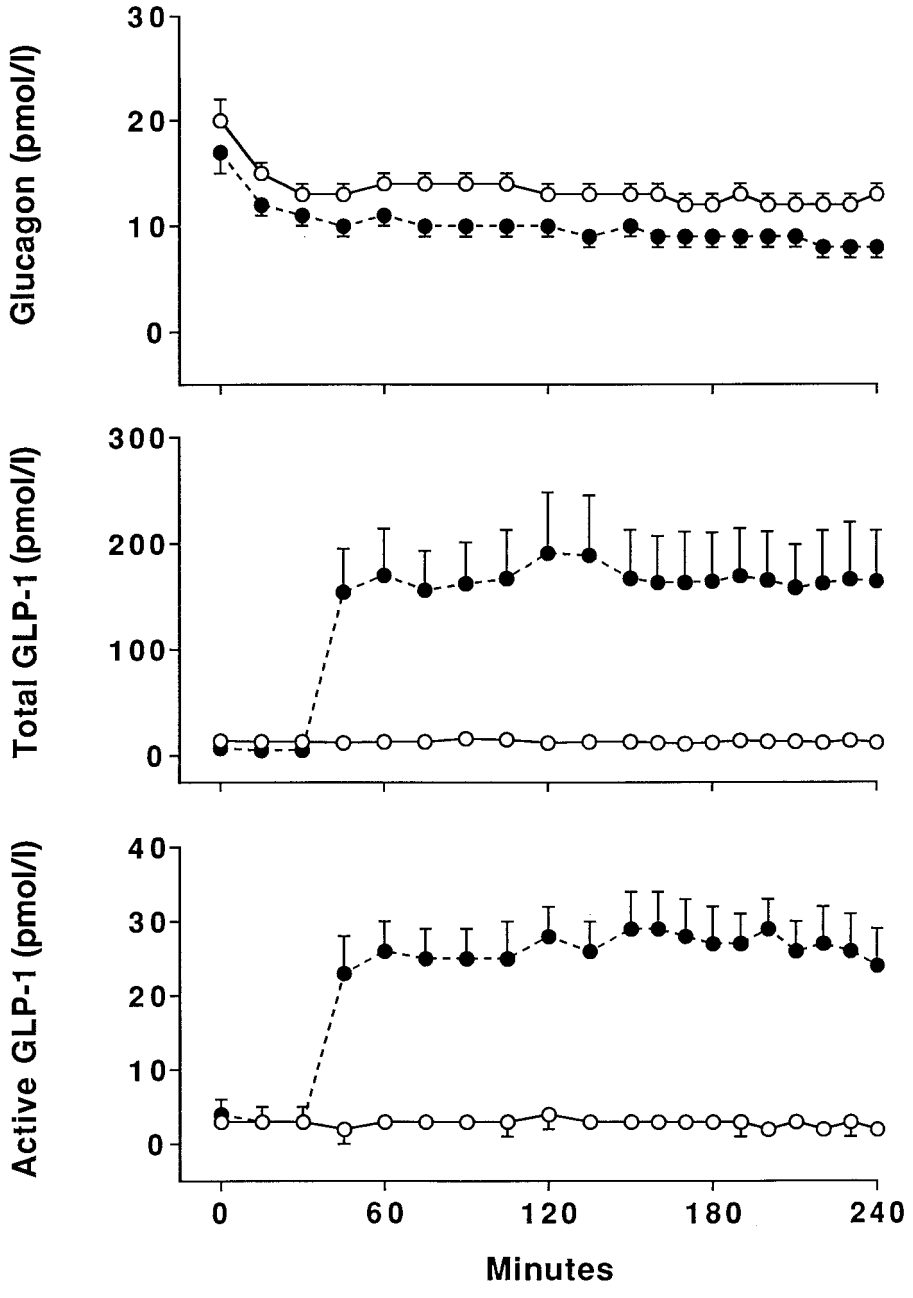

Figure 2—*Glucagon and total and active GLP-1 values during the glucose clamp studies. ○, Control study; ●, GLP-1 study.*

Results were compared using Student's *t* test for paired samples and analysis of variance (ANOVA) as appropriate. $P < 0.05$ was considered significant in all analyses.

RESULTS — Glucose, insulin, glucagon, and GLP-1 values during the study are shown in Figs. 1 and 2 and in Table 1. Basal, 150–180 min, and 210–240 min glucose, insulin, and glucagon values were not different between the control and GLP-1 studies. In both the control and the GLP-1 studies, 150–180 min glucose values were significantly higher than basal, and there was a further significant increase between the 150–180 and the 210–240 min time periods ($P < 0.05$ by ANOVA). However, insulin values were not significantly different at any time point in either study. As expected, both active and total GLP-1 values were higher during the GLP-1 study at both time points ($P < 0.0001$).

R_a and R_d values are shown in Table 1. Basal, 150–180 min, and 210–240 min R_a values were not different between studies. R_d values from 150 to 180 min were similar in both studies. However, 210–240 min R_d values were significantly higher in the GLP-1 study ($P < 0.0001$) (Table 2 and Fig. 3). Peripheral glucose effectiveness was also greater in the GLP-1 study (control study: 0.95 ± 0.34 ml · kg^{-1} · min^{-1}; GLP-1 study: 1.76 ± 0.40 ml · kg^{-1} · min^{-1}; $P < 0.05$).

CONCLUSIONS — Numerous studies have evaluated NIMGU or glucose effectiveness in patients with diabetes, and the results have been conflicting (8). However, it is generally agreed that NIMGU represents an important component of overall glucose disposal in these patients, and interventions that enhance NIMGU are likely to result in reduction of blood glucose levels. As a result, several studies have evaluated strategies that could potentially augment NIMGU. Lowering free fatty acid levels, exercise conditioning, anabolic steroids, and certain oral hypoglycemic agents have all been reported to enhance glucose effectiveness in younger patients (8,17,24–26).

GLP-1 augments insulin release, inhibits glucagon secretion, and delays gastric emptying in type 2 diabetic subjects (12,13,27,28). Therefore, GLP-1 is considered a potentially promising agent for the treatment of type 2 diabetes (29). It has been suggested that GLP-1 might also enhance non–insulin-mediated glucose disposal, but results in normal subjects and diabetic patients have not provided definitive answers (8). D'Alessio et al. (14,15) reported that the peptide enhanced glucose effectiveness in young nondiabetic subjects, whereas Toft-Nielsen et al. (16) did not. Vella et al. (18) found that GLP-1 did not enhance insulin-independent glucose disposal in middle-aged patients with type 2 diabetes.

In the last few years, we have systematically evaluated the metabolic profile of middle-aged and elderly patients with diabetes, and we have demonstrated that diabetes in the elderly appears to be metabolically distinct from younger patients (30). Thus, findings in younger patient populations cannot automatically be extrapolated to the aged. Normal physiologic aging is characterized by a defect in NIMGU under basal conditions but a normal response during hyperglycemia

Table 1—*Glucose, hormone, R_a, and R_d values in the control and GLP-1 studies*

	Basal		150–180 min		210–240 min	
	Control	GLP-1	Control	GLP-1	Control	GLP-1
Glucose (mmol/l)	7.1 ± 0.3	7.3 ± 0.4	8.8 ± 0.3	8.5 ± 0.4	11.2 ± 0.1	11.1 ± 0.1
Insulin (pmol/l)	113 ± 17	113 ± 12	114 ± 16	107 ± 14	126 ± 17	131 ± 16
Glucagon (pmol/l)	20 ± 2.0	17 ± 2.0	13 ± 1.0	10 ± 1.0	12 ± 1.0	9 ± 1.0
Total GLP (pmol/l)	14 ± 3.0	7.0 ± 2.0	12 ± 2.0	164 ± 46*	13 ± 2.0	162 ± 48*
Active GLP (pmol/l)	3.0 ± 1.0	4.0 ± 2.0	3.0 ± 1.0	28 ± 5.0*	3.0 ± 1.0	26 ± 5.0*
R_a (mg · kg^{-1} · min^{-1})	1.90 ± 0.11	1.96 ± 0.11	0.65 ± 0.20	0.53 ± 0.17	0.61 ± 0.18	0.39 ± 0.1
R_d (mg · kg^{-1} · min^{-1})	1.90 ± 0.11	1.96 ± 0.11	2.08 ± 0.13	2.06 ± 0.15	2.52 ± 0.19	2.90 ± 0.1

Data are means ± SEM. *$P < 0.0001$ for control vs. GLP-1 study.

(2,9,10). This defect in NIMGU at basal glucose levels is accentuated in elderly patients with diabetes, implying a combined effect of aging and diabetes on NIMGU in the central nervous system, where ~70% of basal NIMGU occurs (1,5,6). In contrast to healthy elderly subjects, elderly patients with type 2 diabetes also have defects in NIMGU during hyperglycemia, suggesting there is also an abnormal response of muscle to glucose in this patient population (11). Therefore, interventions that enhance NIMGU may be of great therapeutic relevance to the elderly.

The purpose of this study was to specifically evaluate the effect of GLP-1 on NIMGU in elderly patients with diabetes. We found that GLP-1 resulted in an ~15% increase in glucose disposal. In obese elderly diabetic patients, we have previously demonstrated that high physiologic insulin levels increase glucose disposal by ~90% above basal (30). Viewed in the context of overall glucose disposal rates, the increase in glucose disposal in response to GLP-1 would be expected to have a modest clinical benefit. Further studies are needed to test the long-term utility of this peptide in elderly patients in the clinical setting.

We compared our findings with a recent study (18) that found no effect of GLP-1 on NIMGU in middle-aged subjects with diabetes. Vella et al. (18) administered glucose and insulin in a manner that replicated that which occurs during a meal. GLP-1 was infused along with glucose and insulin. The investigators found no effect of GLP-1 on glucose disposal or hepatic glucose production. The discrepancy between the studies may be a result of the age of the subjects and the experimental design. In the study of Vella et al. (18), insulin levels were higher and glucose levels were lower than in our studies, and it is possible that GLP-1 may have differing effects on glucose disposal at different levels of glucose and insulin. In addition, Vella et al. (18) used a lower infusion rate of GLP-1.

Several methodologic concerns need to be addressed. There was a significant increase in glucose values between basal and 150–180 min. Although glucose values were well matched between studies, it is possible that this modest increase in glucose may have played a role in the change in R_d seen at 210–240 min. Although there were no statistically significant differences, insulin values tended to be higher in the control study from 60 to 180 min and higher in the GLP-1 study from 180 to 240 min. Insulin values also had a tendency to increase in each study over time. These subtle changes in insulin values may in part explain the differences, or lack thereof, in R_d that we were able to demonstrate. Because of the gradual increase in insulin levels, it is possible that part of the effect of GLP-1 in this study may have been to enhance insulin-mediated rather than glucose-mediated glucose disposal. However, because insulin-mediated glucose uptake has an ED_{50} (median effective dose) of ~500 pmol/l in normal young subjects and because diabetes in elderly subjects is characterized by insulin resistance (9), we believe that these insulin levels are too low to have any significant effect on glucose disposal. Supporting the concept that residual insulin does not contribute to glucose disposal at these levels, Del Prato et al. (31) found no difference in glucose uptake at basal insulin levels or during insulinopenia. In addition, our findings were confirmed when we calculated glucose effectiveness, which is the change in glucose uptake for the change in glucose level while the insulin level is held constant. Thus, we believe our data are reflective of alterations in NIMGU and not alterations in insulin action. Previous studies have shown that tritiated glucose infusions can result in underestimation of glucose disposal rates and negative hepatic glucose values when insulin values and glucose disposal rates are high. This problem can be corrected by using the "Hot Ginf" technique (32). We elected not to use this technique in our study because insulin levels were suppressed, glucose disposal rates were low, and the "Hot Ginf" technique has not been validated for the hyperglycemic clamp in humans. We also chose not to replace glucagon in these studies. Glucagon infusion could have increased glucose or insulin levels to varying degrees in each study. This would have made it difficult to compare rates of NIMGU at similar glucose and insulin values. In addition, even though GLP-1 has been reported to suppress glucagon secretion, there was no significant difference in glucagon levels between the two experiments.

Table 2—*Individual 210–240 min R_d values (mg · kg^{-1} · min^{-1}) in the control and GLP-1 studies*

Subject no.	Control	GLP-1
1	2.38	2.78
2	3.44	3.48
3	1.82	2.17
4	3.24	3.55
5	3.29	3.74
6	2.14	3.01
7	2.08	2.64
8	2.02	2.39
9	2.28	2.3
10	2.50	2.95
Mean ± SEM	2.52 ± 0.19	2.90 ± 0.17*

*$P < 0.0001$ for control vs. GLP-1 study.

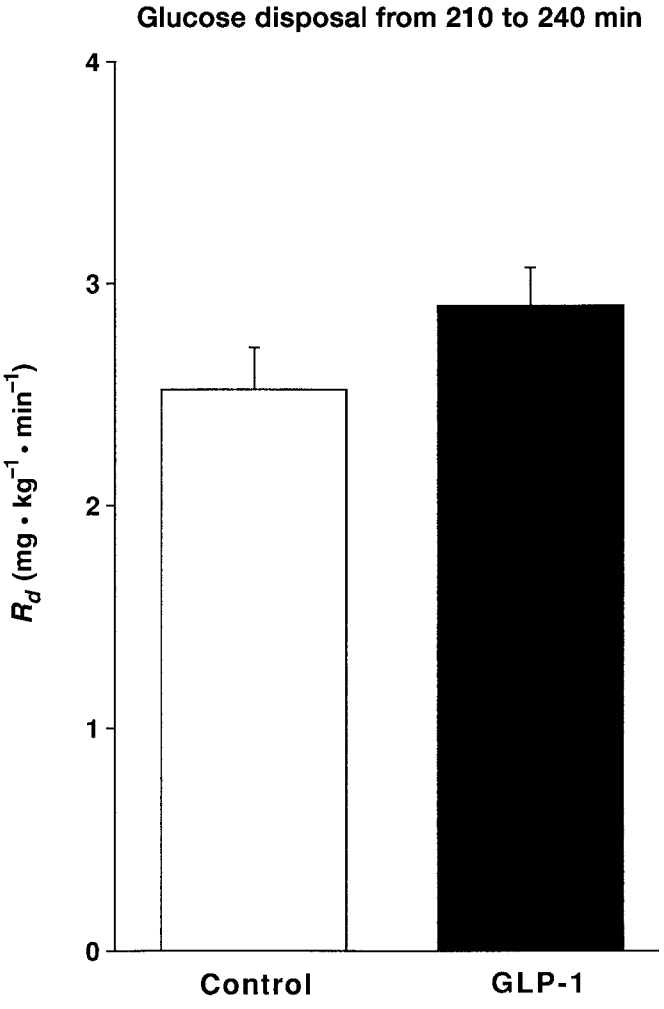

Figure 3—R_d *values during the glucose clamp studies.* $P < 0.0001$ *for control vs. GLP-1 study.*

The levels of active GLP-1 were substantially lower than total GLP-1. The biologic effect GLP-1 is partly regulated through metabolism by dipeptidyl peptidase IV (DPIV) (33), an enzyme that cleaves the first two amino acids from both gastric inhibitory polypeptide (GIP) and GLP-1 (34–36). There is increasing interest in the use of inhibitors of DPIV as a treatment for glucose intolerance and diabetes because these agents are thought to increase the activity of incretin hormones (29). Our data suggest that these inhibitors have the potential to greatly enhance the therapeutic effect of GLP-1 in the elderly.

We conclude that GLP-1 may partially reverse the defect in NIMGU that occurs in elderly patients with diabetes. These data should form the basis for further clinical trials that are designed to assess the effectiveness of this peptide in the elderly.

Acknowledgments— This study was supported by a grant from the Canadian Diabetes Association (in honor of Irene Elizabeth Paget Seath) and in part by a grant from the Medical Research Council of Canada. We gratefully acknowledge the support of the Alan McGavin Geriatric Medicine Endowment of the University of British Columbia and the Jack Bell Geriatric Endowment Fund at Vancouver General Hospital.

The authors acknowledge Christine Lockhart, Gail Chin, Norman Hodges, and Rosemary Torressani for providing technical assistance.

References

1. Baron AD, Brechtel G, Wallace P, Edelman SU: Rates and tissue sites of non-insulin- and insulin-mediated glucose uptake in humans. *Am J Physiol* 255: E769–E774, 1988
2. Meneilly GS, Elahi D, Minaker KL, Sclater AL, Rowe JW: Impairment of non-insulin-mediated glucose disposal in the elderly. *J Clin Endocrinol Metab* 63:566–571, 1989
3. Gottesman I, Mandarino L, Gerich J: Estimation and kinetic analysis of insulin-independent glucose uptake in human subjects. *Am J Physiol* 244:E632–E635, 1983
4. Baron AD, Kolterman OG, Bell J, Mandarino LJ, Olefsky JM: Rates of noninsulin-mediated glucose uptake are elevated in type II diabetic subjects. *J Clin Invest* 76:1782–1788, 1985
5. Huang SC, Phelps ME, Hoffman EJ, Sideris K, Selin CJ, Kuhl DE: Noninvasive determination of local cerebral metabolic rate of glucose in man. *Am J Physiol* 238: E69–E82, 1980
6. Ferranini E, Smith JD, Cobelli C, Toffolo G, Pilo A, DeFronzo RA: Effect of insulin on the distribution and disposition of glucose in man. *J Clin Invest* 76:357–364, 1985
7. Capaldo B, Santoro D, Riccardi G, Perotti N, Sacca L: Direct evidence for a stimulatory effect of hyperglycemia per se on peripheral glucose disposal in type II diabetes. *J Clin Invest* 77:1285–1290, 1986
8. Best JD, Kahn SE, Ader M, Watanabe RM, Ni TC, Bergman RN: Role of glucose effectiveness in the determination of glucose tolerance. *Diabetes Care* 19:1018–1030, 1996
9. Chen M, Bergman RN, Pacini G, Porte D: Pathogenesis of age-related glucose intolerance in man: insulin resistance and decreased beta-cell function. *J Clin Endocrinol Metab* 60:13–20, 1985
10. Fink RI, Wallace P, Olefsky JM: Effect of aging on glucose-mediated glucose disposal and glucose transport. *J Clin Invest* 77:2034–2041, 1986
11. Forbes A, Elliott T, Tildesley H, Finegood D, Meneilly GS: Alterations in non–insulin-mediated glucose uptake in the elderly patient with diabetes. *Diabetes* 47:1915–1919, 1998
12. Orskov C: Glucagon-like peptide-1: a new hormone of the entero-insular axis. *Diabetologia* 35:701–711, 1992
13. Kreymann B, Ghatei MA, Williams G, Bloom SR: Glucagon-like peptide-1 7–36: a physiologic incretin in man. *Lancet* ii: 1300–1304, 1987
14. D'Alessio DA, Prigeon RL, Ensinck JW: Enteral enhancment of glucose disposition by both insulin-dependent and insulin-independent processes: a physiological role of glucagon-like peptide I. *Diabetes* 44:1433–1437, 1995
15. D'Alessio DA, Kahn SE, Leusner CR, Ensinck JW: Glucagon-like peptide 1 enhances glucose tolerance both by stimulation of insulin release and by increasing insulin-independent glucose disposal.

J Clin Invest 93:2263–2266, 1994
16. Toft-Nielsen M, Madsbad S, Holst JJ: The effect of glucagon-like peptide I (GLP-I) on glucose utilization in healthy subjects depends on the pancreatic glucoregulatory hormones. *Diabetes* 45:552–556, 1996
17. Gutniak MK, Junnti-Berggren L, Hellstrom PM, Guenifi A, Holst JJ, Etendi C: Glucagon-like peptide I enhances the insulinotropic effect of glibenclamide in NIDDM patients and in the perfused rat pancreas. *Diabetes Care* 19:857–863, 1996
18. Vella A, Shah P, Basu R, Basu A, Holst JJ, Rizza RA: Effect of glucagon-like peptide 1(7–36) amide on glucose effectiveness and insulin action in people with type 2 diabetes. *Diabetes* 49:611–617, 2000
19. Andres R, Swerdloff R, Pozefsky T, Coleman D: Manual feedback technique for control of glucose concentration. In *Automation in Analytic Chemistry*. Skeggs LT Jr, Ed. New York, P. Medaid, 1966, p. 486–501
20. McGuire EAH, Helderman JH, Tobin JD, Andres JD, Berman M: Arterial versus venous sampling on analysis of glucose kinetics in man. *J Appl Physiol* 41:565–567, 1976
21. Hother-Nielsen O, Beck-Nielsen H: Determination of basal glucose production rate in patients with type II diabetes mellitus using primed continuous 33 h glucose infusion. *Diabetologia* 33:603–610, 1990
22. Krentz AJ, Boyle PJ, MacDonald LM, Schade DS: Octreotide: a long-acting inhibitor of endogenous hormone secretion for human metabolic investigations. *Metabolism* 43:24–31, 1994
23. Ryan AS, Egan JM, Habener JF, Elahi D: Insulinotropic hormone glucagon-like peptide-1-(7–37) appears not to augment insulin-mediated glucose uptake in young men during euglycemia. *J Clin Endocrinol Metab* 83:2399–2404, 1998
24. Sheu W, Jeng CY, Fuh MM, Chen YD, Reaven GM: Effect of glipizide treatment on response to an infused glucose load in patients with NIDDM. *Diabetes Care* 18: 1582–1587, 1995
25. Riccio A, Lisato G, Kreutzenberg SU, Marchetto S, Turrin M, Tiengo A, DellPrato S: Gliclazide potentiates suppression of hepatic glucose production in non-insulin-dependent diabetic patients. *Metabolism* 45:1196–1202, 1996
26. Hobbs CJ, Jones RE, Plymate SR: Nandrolone, a 19-nortestosterone, enhances insulin-independent glucose uptake in normal men. *J Clin Endocrinol Metab* 81: 1582–1585, 1996
27. Nathan DM, Schreiber E, Fogel H, Mojsov S, Habener JF: Insulinotropic action of glucagon-like peptide-I-(7–37) in diabetic and nondiabetic subjects. *Diabetes Care* 15:270–276, 1992
28. Elahi D, McAloon-Dyke M, Fukagawa NK, Meneilly GS, Sclater AL, Minaker KL, Habener JF, Andersen DK: The insulinotropic actions of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1(7–37) in normal and diabetic subjects. *Regul Pept* 51:63–74, 1994
29. Holst JJ, Deacon CF: Inhibition of the activity of dipeptidyl-peptidase IV as a treatment for type 2 diabetes. *Diabetes* 47: 1663–1670, 1998
30. Meneilly GS, Tessier D: Diabetes in the elderly: In *Contemporary Endocrinology, Endocrinology of Aging*. Morley JE, van den Berg L, Eds. Totowa, NJ, Humana, 2000, p. 181–204
31. Del Prato S, Riccio A, Kreutzenberg S, Dorella M, Tiengo A, DeFronzo RA: Basal plasma insulin levels exert a qualitative but not quantitative effect on glucose-mediated glucose uptake. *Am J Physiol* 268: E1089–E1095, 1995
32. Finegood DT, Bergman RN, Vranic M: Modeling error and apparent isotope discrimination confound estimation of endogenous glucose production during euglycemic glucose clamps. *Diabetes* 37: 1025–1034, 1988
33. Demuth H-U, Heins J: Catalytic mechanism of dipeptidyl peptidase IV. In *Dipeptidyl Peptidase IV (CD 26) in Metabolism and the Immune Response*. Fleischer B, Ed. Washington, DC, R.G. Landes Biochemical, 1995, p. 1–37
34. Mentlein R, Gallwitz B, Schmidt WE: Dipeptidyl peptidase IV hydrolyzes gastric inhibitory peptide, GLP-1(7–36), peptide histidine methionine and is responsible for their degradation in human serum. *Eur J Biochem* 214:829–835, 1993
35. Kieffer TJ, McIntosh CHS, Pederson RA. Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 in vitro and in vivo by dipeptidyl peptidase IV. *Endocrinology* 136:3585–3596, 1995
36. Pauly RP, Rosche F, Wermann M, McIntosh CHS, Pederson RA, Demuth H-U: Investigation of glucose-dependent insulinotropic polypeptide-(1–42) and glucagon-like peptide-1-(7–36) degradation in vitro by dipeptidyl peptidase IV using matrix-assisted laser desorption/ionization-time of flight mass spectrometry: a novel kinetic approach. *J Biol Chem* 271:23222–23229, 1996