Enteral Enhancement of Glucose Disposition by Both Insulin-Dependent and Insulin-Independent Processes

A Physiological Role of Glucagon-Like Peptide I

David A. D'Alessio, Ronald L. Prigeon, and John W. Ensinck

Glucagon-like peptide I (GLP-I)(7–36) amide is secreted by intestinal L-cells in response to food ingestion. GLP-I is a potent insulin secretagogue and also inhibits glucagon release. In addition, when given to humans in pharmacological amounts, GLP-I increases glucose disposal independent of its effects on islet hormone secretion. To test the hypothesis that this extrapancreatic effect of GLP-I on glucose disposition is present at physiological levels of GLP-I, we performed intravenous glucose tolerance tests (IVGTTs) 1 h after the following interventions: *1*) the ingestion of 50 g fat to stimulate GLP-I secretion or the ingestion of water as a control and *2*) infusion of GLP-I to attain physiological levels or a control infusion of saline. The results of the IVGTTs were analyzed using the minimal model technique to determine the insulin sensitivity index (S_I) and indexes of insulin-independent glucose disposition, glucose effectiveness at basal insulin (S_G), and glucose effectiveness at zero insulin (GEZI), as well as the glucose disappearance constant (k_g) and the acute insulin response to glucose (AIR_g). These parameters were compared between conditions of elevated circulating GLP-I and control conditions. After ingestion of fat and infusion of synthetic hormone, plasma GLP-I increased to similar levels; GLP-I did not change with water ingestion or saline infusion. Elevated levels of GLP-I, whether from fat ingestion or exogenous infusion, caused increased glucose disappearance (k_g: fat versus water 2.67 ± 0.2 vs. 1.72 ± 0.2, $P < 0.001$; GLP-I versus saline 2.42 ± 0.2 vs. 1.96 ± 0.2 %/min, $P = 0.045$), insulin secretion (AIR_g: fat versus water 427 ± 50 vs. 284 ± 41, $P = 0.001$; GLP-I versus saline 376 ± 65 vs. 258 ± 16 pmol/l, $P = 0.03$), and glucose effectiveness (S_G: fat versus water 2.5 ± 0.1 vs. 1.8 ± 0.2, $P = 0.001$; GLP-I versus saline 2.5 ± 0.2 vs. 1.8 ± 0.2%/min, $P = 0.014$; GEZI: fat versus water 1.9 ± 0.2 vs. 1.3 ± 0.2%/min, $P = 0.003$; GLP-I versus saline 1.9 ± 0.2 vs. 1.3 ± 0.2, $P = 0.006$) but no difference in insulin sensitivity. These results suggest that GLP-I, released after meals, promotes glucose assimilation both by augmenting insulin secretion and through a separate effect to increase glucose uptake and/or inhibit hepatic glucose output. *Diabetes* 44:1433–1437, 1995

From the Division of Endocrinology, Metabolism and Nutrition, Department of Medicine, University of Washington and Veterans Affairs Medical Center, Seattle, Washington.

Address correspondence and reprint requests to Dr. D.A. D'Alessio, Dept. of Medicine, RC-14, University of Washington, 1959 Pacific Ave., Seattle, WA 98195.

Received for publication 31 May 1995 and accepted in revised form 10 August 1995.

AIR_g, acute insulin response to glucose; BIE, basal insulin effect; GI, gastrointestinal; GEZI, glucose effectiveness at zero insulin; GIP, gastric inhibitory polypeptide; GLP-I, glucagon-like peptide I; IVGTT, intravenous glucose tolerance test; K_g, glucose disappearance constant; RIA, radioimmunoassay; S-28, somatostatin 28; S_G, glucose effectiveness at basal insulin; S_I, sensitivity index.

The roles of gastrointestinal (GI) hormones in mediating the disposition of absorbed nutrients have been the focus of investigation since early in this century. Central to this formulation is the action of GI peptides to augment insulin secretion in concert with the increasing levels of plasma nutrients during the assimilation of a meal: the incretin effect (1–3). Two GI peptides are generally accepted as being physiological incretins: gastric inhibitory polypeptide (GIP), located in K-cells in the upper small intestine, and glucagon-like peptide I (GLP-I)(7–36) amide, produced by L-cells in the distal gut. Both are released in response to carbohydrate and fat meals and have a glucose-dependent insulinotropic effect (3,4). GLP-I also inhibits glucagon release (5,6). Synergistic insulinotropic activity between GIP and GLP-I has been demonstrated both in isolated rat pancreas and in humans (7,8), and it is probable that these two incretins act together to coordinate islet function during nutrient absorption.

Recent studies indicate that GLP-I promotes glucose disposition independent of insulin. Gutniak et al. (9) clamped insulin levels in a group of type I diabetic subjects and showed that glucose disappearance was greater during infusions of pharmacological amounts of GLP-I than during saline administration. We subsequently found that healthy humans given a supraphysiological dose of GLP-I during intravenous glucose tolerance tests (IVGTTs) had a 50% increase in glucose effectiveness, a measure of the insulin-independent component of glucose disappearance (10). Plasma glucagon concentrations were not significantly changed by GLP-I in this experiment, indicating that this hormone did not contribute to the observed increase in glucose disappearance (10). These results imply that GLP-I may have a direct effect on the processes involved in glucose metabolism, i.e., stimulation of glucose uptake and/or suppression of hepatic glucose output. Recent work supporting this contention (11,12) includes the detection of GLP-I receptor gene expression in liver, muscle, and adipose cells; GLP-I receptor activity in adipocytes and preadipocytes (12); GLP-I-stimulated increases in glycogen synthesis in rat soleus muscle and isolated hepatocytes; and augmented glucose transport in an adipocyte cell line treated with GLP-I (12–14). In vivo, the putative extrapancreatic action of GLP-I has only been demonstrated with pharmacological amounts of the peptide, and it is unclear whether or not physiological concentrations of the hormone have similar effects on glu-

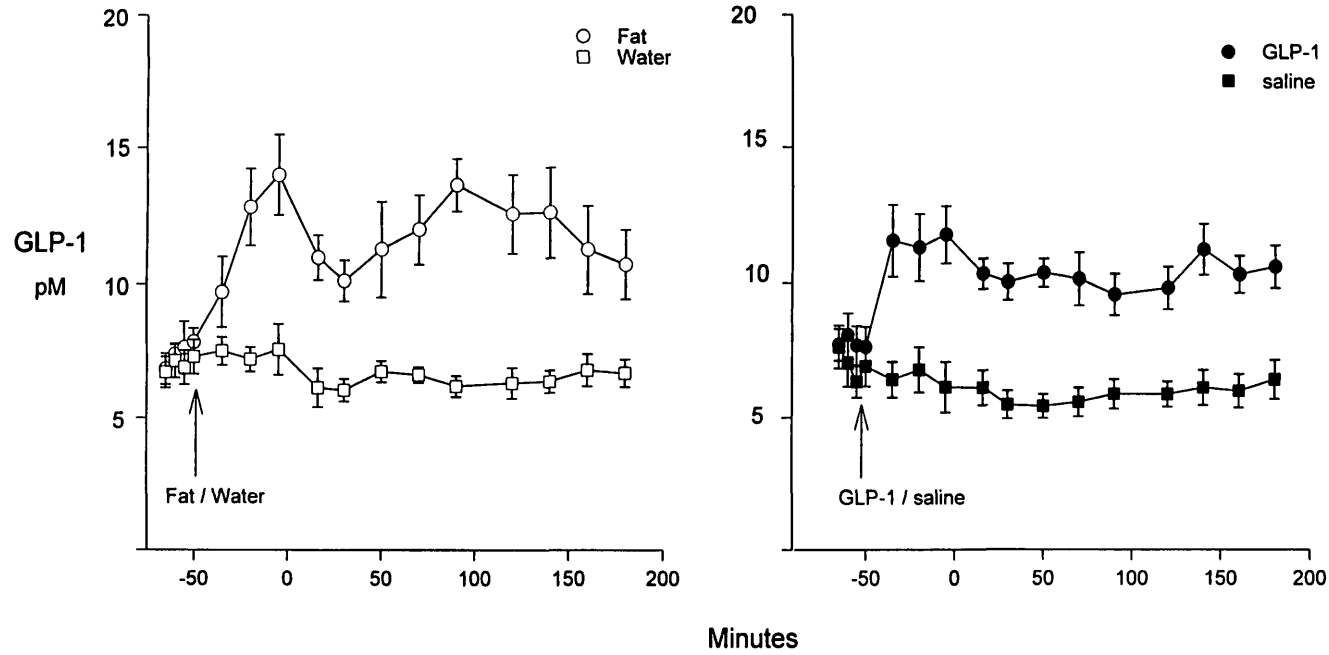

FIG. 1. Circulating concentrations of GLP-I in healthy humans before and after ingestion of 50 g fat or water (n = 10) and before and after intravenous administration of synthetic GLP-I or saline (n = 10). Data are presented as means ± SE.

cose disposal. We report herein that postprandial levels of GLP-I, achieved by either exogenous or endogenous means, are also associated with increased insulin-independent glucose disposition, supporting a novel physiological role for GLP-I.

RESEARCH DESIGN AND METHODS

Seventeen healthy individuals (5 women and 12 men), ages 22–54 years, were recruited. They had stable body weights within 15% of ideal body weight and had no personal or family history of diabetes. After the nature of the study had been explained, subjects signed informed consent forms that had been approved by the institutional human subjects committee. Volunteers were admitted to the Clinical Research Center at the University of Washington, Seattle, WA, on two separate mornings after 10- to 12-h fasts to participate in one of two protocols. The fat/water protocol involved oral intake of either 50 g fat, in the form of heavy cream, or an equivalent amount of water. The GLP-I/saline protocol consisted of intravenous administration of either synthetic GLP-I (prepared as described previously [10] at the Howard Hughes Medical Institute, University of Washington) at 15 pmol · kg^{-1} · h^{-1} or 150 mmol/l NaCl. This dose of GLP-I raises plasma GLP-I concentrations to postprandial levels (D.A.D., J.W.E., unpublished observations). Ten subjects participated in each protocol, and the studies were balanced such that the control study was performed first in half of the subjects. The paired studies were separated by at least 1 week in the men and were done at similar points in the menstrual cycle in the women. After the ingestion of fat or water or the infusion of GLP-I or saline, blood was sampled at 10-min intervals for 1 h. Then a bolus of glucose (11.4 g/m^2) was given intravenously as the initiation of a modified frequently sampled intravenous glucose tolerance test (IVGTT) (15); 20 min after the glucose infusion, tolbutamide (125 mg/m^2) was administered intravenously and blood samples were collected for an additional 160 min as previously described.

Blood was collected into heparinized tubes for insulin and glucose analyses and into EDTA/aprotinin (50 mmol · l^{-1} · 500 IU^{-1} · ml^{-1}) for the measurement of GLP-I. After immediate centrifugation, the separated plasma was stored at −20°C. Glucose was measured by a glucose oxidase method, and insulin concentrations were determined by specific radioimmunoassay (RIA) (16). GLP-I was measured by RIA using antiserum 89390 (donated by Dr. J.J. Holst, Paanum Institute, Copenhagen), as described by Orskov and Holst (17), in ethanol extracts of plasma. Antiserum 89390 recognizes the COOH-terminal amidated arginine of GLP-I and thus is specific for the amidated GLP-I species, which includes GLP-I(7–36) amide, the predominant bioactive GLP-I species (18).

Fasting insulin and glucose levels were designated as the means of four samples taken before the fat/water and GLP-I/saline treatments. The acute insulin response to glucose (AIR_g) was computed as the mean increase in insulin over the first 10 min after administration of the intravenous glucose minus the baseline insulin; the baseline value was taken as the average of values sampled 0, 10, and 20 min before the injection of glucose. The glucose disappearance constant (k_g) was derived from the slope of the natural logarithm of the five glucose samples taken 10–19 min after administration of glucose. The insulin and glucose data were analyzed using the minimal model of glucose kinetics (19) to derive indexes of insulin sensitivity (S_I) and glucose effectiveness (S_G). Since S_G is the glucose effectiveness at basal insulin levels, it includes an insulin-dependent component, or basal insulin effect (BIE), which is computed by multiplying the baseline insulin level by the insulin sensitivity index (S_I). Glucose effectiveness at zero insulin (GEZI), a true measure of insulin-independent glucose disposition, was calculated by subtracting the BIE from the S_G (20). The minimal model allows separation of glucose disposition into insulin-dependent and insulin-independent components at any time point during the IVGTT (21). To determine the relative contributions of insulin-dependent and insulin-independent processes to glucose disappearance (k_g), mean values of these terms were computed from the samples obtained from time 10–19 min. Comparisons were made using the t test or the rank-sum test for paired data; two-tailed analyses were done except where noted. Data are presented as means ± SE.

RESULTS

Fasting GLP-I concentrations in the subjects were similar on the days they received fat, water, GLP-I, or saline (7.4 ± 0.6, 7.2 ± 0.6, 7.4 ± 0.8, and 7.5 ± 0.8 pmol/l, respectively). After ingestion of cream, GLP-I concentrations rose within 15–20 min, and levels remained elevated at ~13 pmol/l throughout the 4-h study (Fig. 1). Infusion of GLP-I achieved a similar increase in the circulating GLP-I concentrations to 11–12 pmol/l that was maintained throughout the study (Fig. 1). GLP-I levels did not change significantly from the fasting values when the subjects received water or saline.

In the 1st h after the ingestion of fat, insulin levels increased from 60.6 ± 9.0 to 96 ± 15.6 pmol/l ($P < 0.01$) and glucose levels decreased from 4.8 ± 0.01 to 4.6 ± 0.01 mmol/l ($P < 0.05$) compared with preprandial values. In contrast, there were no differences between fasting and pre-IVGTT

TABLE 1
Parameters of intravenous glucose tolerance in groups of 10 healthy humans ingesting 50 g fat or water or receiving intravenous GLP-1 or saline

	Fat	Water	*P* value	GLP-1	Saline	*P* value
k_g ($\cdot 10^{-2} \cdot min^{-1}$)	2.67 ± 0.19	1.72 ± 0.17	<0.001	2.42 ± 0.19	1.96 ± 0.21	0.045*
AIR_g (pmol/l)	427 ± 50	284 ± 41	<0.001	376 ± 65	258 ± 16	0.03
S_I ($pmol \cdot l^{-1} \cdot min^{-1} \cdot 10^{-5}$)	9.5 ± 2.7	7.5 ± 1.4	0.15	8.2 ± 1.3	8.4 ± 1.3	>0.3
S_G ($\cdot 10^{-2} \cdot min^{-1}$)	2.5 ± 0.1	1.8 ± 0.2	0.001	2.5 ± 0.2	1.8 ± 0.2	0.014
GEZI ($\cdot 10^{-2} \cdot min^{-1}$)	1.9 ± 0.1	1.3 ± 0.2	0.003	1.9 ± 0.2	1.3 ± 0.2	0.006

Data are means ± SE. * One-tailed analysis.

insulin and glucose concentrations after GLP-I infusion, saline infusion, or water intake.

Intravenous glucose tolerance was increased when circulating GLP-I levels were elevated, as reflected in the higher k_g on the days the subjects drank cream than on the days they drank water, and during the GLP-I compared with saline infusions (Table 1). The AIR_g was significantly greater during increases in either endogenous or exogenous GLP-I (Table 1). Therefore, a portion of the increase in k_g was accounted for by incretin-stimulated insulin secretion.

A second factor in the greater k_g observed with experimental elevation in circulating GLP-I was augmented glucose effectiveness. This was detected whether glucose effectiveness was calculated at basal insulin levels (S_G; Table 1) or at zero insulin (GEZI; Table 1), a true measure of insulin-independent glucose disposition. Increasing the circulating concentrations of GLP-I, either by infusion or by nutrient stimulation, had no effect on insulin sensitivity, since there was no difference in S_I between the paired experiments (Table 1). The contribution of insulin-independent glucose disposition to k_g did not differ between the control and increased GLP-I conditions (fat versus water 39.0 ± 3.5 vs. 47.9 ± 2.9%, $P = 0.3$; GLP-I versus saline 44.8 ± 5.1 vs. 41.9 ± 5.6%, $P = 0.135$).

DISCUSSION

This study demonstrates that GLP-I at concentrations of the hormone achieved after nutrient intake promotes glucose disposition both by enhancement of insulin secretion and by insulin-independent processes. As reflected in the minimal model parameter GEZI, insulin-independent glucose disposition in humans increased coincident with elevations in circulating endogenous GLP-I levels stimulated by a fat meal or after infusion of synthetic hormone to achieve an approximately matching level. The higher rates of insulin-independent glucose disposition were nearly identical whether GLP-I concentrations were increased from endogenous or exogenous sources, suggesting that GLP-I was the causative factor. These data extend our previous observations in humans, which used supraphysiological infusions of GLP-I and are consistent with a physiological role of GLP-I in postprandial glucose homeostasis that is distinct from its ability to stimulate insulin secretion.

Plasma GLP-I immunoreactivity, as measured by the COOH-terminal antisera used in our RIA, is a heterogeneous mix of related peptides that includes the precursor GLP-I(1–36) amide and the metabolite GLP-I(9–36) amide (22,23). Therefore, establishing physiological concentrations of the active GLP-I(7–36) amide with this assay is likely to be somewhat imprecise. However, because GLP-I(7–36) amide is the predominant secreted form of the GLP-I peptides (22), it is reasonable to conclude that the rise in GLP-I immunoreactivity measured after the fat meal reflects increases of this moiety in the circulation. Since it is unlikely that the intravascular metabolism of synthetic GLP-I(7–36) amide varies significantly from the secreted form, the matching of infused GLP-I to postfat levels should have achieved a reasonable approximation of bioactive hormone in both conditions. The concordance of the IVGTT results with these two experimental paradigms supports this inference.

Insulin-independent glucose disposition has been estimated to comprise 40% of the glucose disappearance with intravenous glucose loads (24) and 40–60% of glucose disposition after oral glucose (25). We found a similar relative contribution of glucose effectiveness to glucose disappearance in our studies. There was no difference in the percentage of insulin-independent glucose disposition in the experimental and control studies, indicating that GLP-I augmented intravenous glucose tolerance by increasing insulin-dependent and insulin-independent processes proportionately.

Although Nauck et al. (6) have shown that infusions of GLP-I suppress plasma glucagon levels during hyperglycemic clamps, we could not demonstrate a difference in plasma glucagon concentrations during an IVGTT with GLP-I or saline administration in our previous study (10). We concluded that the augmentation of S_G seen with a GLP-I infusion was independent of the influence of glucagon. Because we infused GLP-I at a rate three times greater than that used in the present study, it is improbable that glucagon levels differed between the saline and GLP-I experiments reported herein. Glucagon secretion has been noted to be minimally altered in humans after a lipid meal (26,27), so it is unlikely that the difference in insulin-independent glucose disposition between the fat and water experiments can be accounted for by glucagon effects.

The mechanisms whereby glucose promotes its own disappearance from the circulation are unclear, but several recent studies have demonstrated that this process is regulated (10,20,28) and thus amenable to the influence of hormones. Heretofore, GLP-I has been the only hormone reported to accelerate insulin-independent glucose disposition. It can be inferred from the expression of the GLP-I receptor gene in a wide variety of tissues (11,12) that GLP-I may affect glucose metabolism at more than one site. Preliminary work in vitro suggests that GLP-I may stimulate glycogen synthesis in hepatocytes and muscle cells (13,14), but the relation of these findings to augmented insulin-independent glucose disposition in vivo has yet to be established. In our previous study, we found that infusion of GLP-I at 45 $pmol \cdot kg^{-1} \cdot h^{-1}$ (subsequently shown to attain supraphysiological plasma concentrations of 25–45 pmol/l [D.A.D., R.L.P., J.W.E., unpublished observations]) was associated

with a 50% increase in S_G (10). Comparable increases in glucose-mediated glucose disposal were achieved in the current study by circulating levels of GLP-I that were much lower. These findings suggest that the enhancement of glucose effectiveness unique to GLP-I occurs at physiological concentrations of the hormone and is likely to be an important factor in the assimilation of ingested carbohydrate.

Intake of fat caused a modest but significant rise in insulin levels and a decrease in glucose concentrations before the IVGTT. These changes were not observed with the matched infusion of GLP-I. The increase in basal insulin output after the fat meal may have been the result of substrate stimulation of the β-cells, since the ingested cream contained 4 g carbohydrate and 3 g protein, or the influence of other enteroinsular factors. The insulinotropic effect of GLP-I has been shown to be glucose dependent in vitro (4), and we previously observed no effect of pharmacological infusions of GLP-I on insulin levels at fasting glycemia (10). Despite the fact that oral intake of fat stimulates the release of a host of GI peptides, including GIP (3), there was little difference in the insulin response to intravenous glucose between the cream ingestion and the GLP-I infusion. While it is plausible that other gut hormones contributed to the augmented AIR_g after the fat meal, the results from the GLP-I/saline protocol confirm the previous observation that GLP-I has a significant role in the incretin effect (6,29). However, since most of the subjects did not participate in both experimental conditions, we cannot directly compare the incretinic effect of GLP-I alone with that of the enterally stimulated GI tract.

We have previously shown that in healthy humans studied 2 h after intake of 50 g fat, the acute insulin response to intravenous arginine or secretin is blunted compared with that elicited after water ingestion (30). We attributed this result to the inhibitory action of somatostatin-28 (S-28) secreted by endocrine cells within the proximal intestine because *1*) circulating concentrations of this hormone peak relatively late after nutrients are consumed and *2*) infusion of synthetic S-28 to postprandial levels caused a similar decrement in stimulated insulin release. The augmentation of AIR_g 1 h after an identical fat meal in the current study implies that the regulatory effects of GI factors on the β-cell vary temporally through the course of meal absorption. That is, incretins released in the early postprandial period promote insulin release while substrate levels are increasing, and signals such as S-28, which damp insulin secretion, predominate in the later phases of digestion as the provision of nutrients from the gut diminishes.

In summary, the current data demonstrate that the circulating concentrations of GLP-I present early after meal ingestion promote glucose disposition independent of insulin consistent with a novel physiological role for this hormone. In addition, the incretinic effect of GLP-I contributes significantly to the augmented insulin response after oral nutrient intake. It is now reasonable to propose that GLP-I release, stimulated by a meal, not only serves to link nutrient absorption with pancreatic β-cell secretion but also signals some of the key target tissues in glucose metabolism separate from its insulinotropic action.

ACKNOWLEDGMENTS

This work was supported by U.S. Public Health Service Grants DK-34397, RR-0037, and DK-02305.

We thank Ellen Laschansky for precise measurements of peptide hormones, Karman Eng for glucose analyses and other technical support, Henry Zebroski for synthesis and purification of GLP-I, and the nurses of the Clinical Research Center at the University of Washington for assistance with the studies.

REFERENCES

1. McIntyre N, Holdsworth CD, Turner DS: Intestinal factors in the control of insulin secretion. *J Clin Endocrinol* 25:1317–1324, 1965
2. Perley MJ, Kipnis DM: Plasma insulin responses to oral and intravenous glucose: studies in normal and diabetic subjects. *J Clin Invest* 46:1954–1962, 1967
3. Creutzfelt W, Ebert R: New developments in the incretin concept. *Diabetologia* 28:565–573, 1985
4. Weir GC, Mojsov S, Hendrick GK, Habener JF: Glucagon like peptide 1 (7–37) actions on endocrine pancreas. *Diabetes* 38:338–342, 1989
5. Komatsu R, Matsuyama T, Namba M, Watanabe N, Itoh H, Kono N, Tarui S: Glucagonostatic and insulinotropic action of glucagonlike peptide 1-(7–36)-amide. *Diabetes* 38:902–905, 1989
6. Nauck MA, Heimesaat MM, Orskov C, Holst JJ, Ebert R, Creutzfeldt W: Preserved incretin activity of glucagon-like peptide 1 (7–36 amide) but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. *J Clin Invest* 91:301–307, 1993
7. Fehmann H-C, Goke B, Goke R, Trautmann ME, Arnold R: Synergistic effect of glucagon-like peptide 1 (7–36) amide and glucose-dependent insulin-releasing polypeptide on the endocrine rat pancreas. *FEBS Lett* 252:109–112, 1989
8. Nauck MA, Bartels E, Orskov C, Ebert R, Creutzfeldt W: Additive insulinotropic effects of exogenous synthetic human gastric inhibitory polypeptide and glucagon-like peptide-1(7–36) amide infused at near-physiological insulinotropic hormone and glucose concentrations. *J Clin Endocrinol Metab* 76:912–917, 1993
9. Gutniak M, Orskov C, Holst J, Ahren B, Efendic S: Antidiabetogenic effect of glucagon-like peptide-1(7–36)amide in normal subjects and patients with diabetes mellitus. *N Engl J Med* 326:1316–1322, 1992
10. D'Alessio DA, Kahn SE, Leusner C, Ensinck J: Glucagon-like peptide 1(7–36 NH2) enhances glucose tolerance both by stimulation of insulin release and by increasing insulin independent glucose disposal. *J Clin Invest* 93:2263–2266, 1994
11. Wheeler MB, Lu M, Dillon JS, Leng X-H, Chen C, Boyd AE: Functional expression of the rat glucagon-like peptide-I receptor, evidence for coupling to both adenyl cyclase and phospholipase-C. *Endocrinology* 133:57–62, 1993
12. Egan JM, Montrose-Rafizadeh C, Wang Y, Bernier M, Roth J: Glucagon-like peptide 1 (7–36)amide enhances insulin-stimulated glucose metabolism in 3T3-LI adipocytes: one of several potential extrapancreatic sites of GLP-I action. *Endocrinology* 135:2070–2075, 1994
13. Valverde I, Morales M, Clemente F, Lopez-Delgado MI, Delgado E, Perea A, Villanueva-Penacarrillo M-L: Glucagon-like peptide 1: a potent glycogenic hormone. *FEBS Lett* 349:313–316, 1994
14. Villaneuva-Penacarrillo ML, Alcantara AI, Clemente F, Delgado E, Valverde I: Potent glycogenic effect of GLP-1 (7–36)amide in rat skeletal muscle. *Diabetologia* 37:1163–1166, 1994
15. Beard JC, Bergman RN, Ward WK, Porte D: The Insulin Sensitivity Index in nondiabetic man. *Diabetes* 35:362–369, 1986
16. Zaharko D, Beck L: Studies of a simplified plasma insulin immunoassay using cellulose powder. *Diabetes* 17:444–457, 1968
17. Orskov C, Holst JJ: Radio-immunoassays for glucagon-like peptides 1 and 2. *Scand J Clin Lab Invest* 47:165–174, 1987
18. Orskov C, Rabenhoj L, Wettergren A, Kofod H, Holst JJ: Tissue and plasma concentrations of amidated and glycine-extended glucagon-like peptide in humans. *Diabetes* 43:535–539, 1994
19. Bergman RN, Phillips L, Cobelli C: Physiologic evaluation of factors controlling glucose tolerance in man. *J Clin Invest* 68:1456–1467, 1979
20. Kahn SE, Klaff LJ, Schwartz MW, Beard JC, Bergman RN, Taborsky GJ, Porte D Jr: Treatment with a somatostatin analog decreases pancreatic B-cell and whole body sensitivity to glucose. *J Clin Endocrinol Metab* 71:994–1002, 1990
21. Pacini G, Bergman RN: MINMOD: a computer program to calculate insulin sensitivity and pancreatic responsivity from the frequently sampled intravenous glucose tolerance test. *Comp Prog Biomed* 23:113–122, 1986
22. Orskov C, Rabenhoj L, Wettergren A, Kofod H, Holst JJ: Tissue and plasma concentrations of amidated and glycine-extended glucagon-like peptide in humans. *Diabetes* 43:535–539, 1994
23. Deacon CF, Johnsen AH, Holst JJ: Degradation of glucagon-like peptide-1 by human plasma in vitro yields an N-terminally truncated peptide that is a major endogenous metabolite in vivo. *J Clin Endocrinol Metab* 80:952–957, 1995
24. Kahn SE, Prigeon RL, McCulloch DK, McKnight B, Schwartz MW, Bergman RN, Palmer JP, Porte D Jr: The contribution of insulin indepen-

dent glucose uptake to intravenous glucose tolerance in healthy human subjects. *Diabetes* 43:587–592, 1994
25. Henriksen JE, Alford F, Handberg A, Vaag A, Ward GM, Kalfas A, Beck-Nielsen H: Increased glucose effectiveness in normoglycemic but insulin-resistant relatives of patients with non-insulin-dependent diabetes mellitus. *J Clin Invest* 94:1196–1204, 1994
26. Bottger I, Dobbs R, Faloona GR, Unger RH: The effects of triglyceride absorption upon glucagon, insulin and gut glucagon-like immunoreactivity. *J Clin Invest* 52:2532–2541, 1973
27. Andrews SS, Lopez SA, Blackard WG: Effect of lipids on glucagon secretion in man. *Metabolism* 24:35–44, 1975
28. Page R, Boolell M, Kalfas A, Sawyer S, Pestell R, Ward G, Alford F: Insulin secretion, insulin sensitivity, and glucose-mediated glucose disposal in Cushing's disease: a minimal model analysis. *Clin Endocrinol* 35:509–517, 1991
29. Kreymann B, Ghatei MA, Williams G, Bloom SR: Glucagon-like peptide-1 7–36: a physiological incretin in man. *Lancet* ii:1300–1303, 1987
30. D'Alessio DA, Sieber C, Beglinger C, Ensinck J: A physiologic role for somatostatin 28 as a regulator of insulin secretion. *J Clin Invest* 84:857–862, 1989