Glucagon-like peptide-1 accelerates the onset of insulin action on glucose disappearance in mice

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Submitted 23 June 2006; accepted in final form 13 February 2007

Thomaseth K, Pavan A, Pacini G, Ahren B. Glucagon-like peptide-1 (GLP-1) accelerates the onset of insulin action on glucose disappearance in mice. Am J Physiol Endocrinol Metab 292:E1808–E1814, 2007. First published February 20, 2007; doi:10.1152/ajpendo.00303.2006.—Glucagon-like peptide-1 (GLP-1) plays a significant role in glucose homeostasis through its incretin effect on insulin secretion. However, GLP-1 also exhibits extrapancreatic actions, and its possible influences on insulin sensitivity are controversial. To study the dynamic action of GLP-1 on insulin sensitivity, we applied advanced statistical modeling methods to study glucose disappearance in mice that underwent intravenous glucose tolerance test with administration of GLP-1 at various dose levels. In particular, the minimal model of glucose disappearance was exploited within a population estimation framework for accurate detection of relationships between glucose disappearance parameters and GLP-1. Minimal model parameters were estimated from glucose and insulin data collected in 209 anesthetized normal mice after intravenous injection of glucose (1 g/kg) alone or with GLP-1 (0.03–100 nmol/kg). Insulin secretion markedly increased, as expected, with increasing GLP-1 dose. However, minimal model-derived indexes, i.e., insulin sensitivity and glucose effectiveness, did not significantly change with GLP-1 dose. Instead, fractional turnover rate of insulin action [P2 = 0.0207 ± 24.3% (min) at zero GLP-1 dose] increased steadily with administered GLP-1 dose, with significant differences at 10.4 nmol/kg (P2 = 0.040 ± 15.5%, P = 0.0046) and 31.2 nmol/kg (P2 = 0.050 ± 29.2%, P = 0.01). These results show that GLP-1 influences the dynamics of insulin action by accelerating insulin action following glucose challenge. This is a novel mechanism contributing to the glucose-lowering action of GLP-1.

Glucagon-like peptide-1 (GLP-1) is under consideration as a pivotal hormone for the treatment of type 2 diabetes mellitus (7). Several mechanisms may explain the antidiabetic action of GLP-1, the most important being the stimulation of insulin secretion and inhibition of glucagon secretion (16). A recent study (4) suggests that GLP-1 also acts by increasing the bioavailability of endogenously secreted insulin through reduction of insulin clearance. However, whether GLP-1, in addition, affects insulin sensitivity is a matter of controversy. In humans, short-term studies (2, 11) have shown no effect in clamp experiments, whereas long-term administrations (21) have indicated improved insulin sensitivity due to improved glucose metabolism. A short-term study in depancreatized dogs (17) reported that GLP-1 enhances insulin-stimulated glucose utilization. In contrast, in a previous study in mice (3), increasing doses of GLP-1 (from 0.03 to 100 nmol/kg) given intravenously along with glucose progressively reduced insulin sensitivity, which was measured as the insulin sensitivity index (SI) obtained by mathematical modeling of glucose and insulin data collected during the intravenous glucose tolerance test (IVGTT). This was interpreted as a direct action of the peptide to inhibit insulin action, which would suggest that, at high doses of GLP-1, a safeguard insulin resistance is induced with the purpose of diminishing the risk of hypoglycemia in the condition of the marked stimulation of insulin secretion. A limitation of this conclusion, however, is due to the implicit assumption that GLP-1 affects the action of insulin on glucose disappearance only through the overall insulin sensitivity, whereas in dynamic experiments, such as IVGTT, the time profile of insulin action also plays an important role (1, 13).

The present study aimed at clarifying the short-term effects of GLP-1 by accurate assessment on how GLP-1 produces time-dependent perturbations in insulin action. For this purpose, advanced statistical modeling techniques, called nonlinear mixed-effects (NLME) population modeling (14), were exploited in the analysis of IVGTT data using the previously validated minimal model of glucose disappearance (12). The population approach makes a more efficient use of experimental information through simultaneous analysis of all available data, yielding more accurate results on within and between individual variability of kinetic parameters and on their relationship with covariates (14).

MATERIALS AND METHODS

Animals. Experimental data from two previous studies (3, 4) were combined and reanalyzed. In particular, a total of 209 nonfasted NMRI mice (Taconic, Ry, Denmark) weighing 20–25 g were used throughout the study. The animals were fed a standard pellet diet and tap water ad libitum. The study was approved by the Animal Ethics Committee of Lund and Malmö.

IVGTT. Experimental protocols are detailed elsewhere (3). Briefly, the mice were anesthetized with an intraperitoneal injection of midazolam (Dormicum, 0.4 mg/mouse; Hoffman-La Roche, Basel, Switzerland) and a combination of fluanison (0.9 mg/mouse) and fentanyl (Hypnorm, 0.02 mg/mouse; Janssen, Beerse, Belgium). Thereafter, a blood sample was taken from the retrobulbar, intraorbital, capillary plexus in heparinized tubes, whereafter D-glucose (1 g/kg; British Drug Houses, Poole, UK) was rapidly injected intravenously in a tail vein either alone or together with synthetic GLP-1 at various dose levels (Peninsula Laboratories Europe, Merseyside, UK). In particular, dose levels (nmol/kg) and corresponding number of animals were 0 (n = 92, control group), 0.03 (n = 4), 0.1 (n = 8), 0.3, (n = 12), 1.0 (n = 8), 3.0 (n = 9), 10.4 (n = 51), 31.2 (n = 9), and 100 (n = 16). The volume load was 10 μl/kg body wt. Additional samples were taken after 1, 5, 10, 20, 30, and 50 min. Following immediate centrifugation, plasma was separated and stored at −20°C until analysis.

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Analysis. Plasma insulin was determined radiioimmunochemically with the use of a guinea pig anti-rat insulin antibody, $^{125}$I-labeled human insulin as tracer, and rat insulin as standard (Linco Research, St. Charles, MO). Free and bound radioactivity was separated by use of an anti-IgG (goat anti-guinea pig) antibody (Linco Research). The sensitivity of the assay is 12 pmol/l, and the coefficient of variation is <3%. Plasma glucose was determined with the glucose oxidase method.

Minimal modeling analysis. Insulin and glucose data from the seven-sample IVGTT were analyzed with the minimal model technique (12) within a population NLME modeling framework (14). In summary, the model assumes first-order glucose kinetics with nonlinear control by insulin and accounts for the effect of insulin and glucose itself on glucose disappearance following exogenous glucose injection. Equations are as follows:

$$\frac{dG(t)}{dt} = -[S_G + X(t)]G(t) + S_G G_b \quad (I)$$

$$\frac{dX(t)}{dt} = -P_2[X(t) - S_I[I(t) - I_b]] \quad (2)$$

where $G(t)$ and $X(t)$ are plasma glucose (mmol/l) and insulin action (min$^{-1}$) associated with insulin in a remote compartment, respectively; $G_b$ and $I_b$ are reference basal concentrations of glucose and insulin; $I(t)$ is plasma insulin concentration (pmol/l); $S_I$ [min$^{-1} \times$ (pmol/l)$^{-1}$] is defined as the ability of insulin to enhance glucose disappearance and inhibit glucose production (5); $S_G$ (glucose effectiveness) (min$^{-1}$) assesses glucose disappearance from plasma per se without any change in dynamic insulin (1); and $P_2$ represents the turnover rate (min$^{-1}$) of insulin action in the remote compartment and determines how much variations in insulin action lag behind deviations from basal of plasma insulin concentrations. Initial conditions in equations 1 and 2 are $G(0) = G_b + D/V_G$, where $D$ is the injected glucose dose (mmol), $V_G$ the distribution volume (l), and $X(0) = 0$. The values assigned to $G_b$ and $I_b$ were the basal concentrations at time 0. The insulin time profile $I(t)$ was obtained by linear interpolation of the insulin concentration measurements. Model parameters were log transformed before estimation to guarantee positive values for back-transformed parameters and to reduce the effects of possible large between-animal variability.

Statistics. Minimal modeling analysis was performed by simultaneously fitting the data of all studied animals with a NLME population approach. Population methods are more dependable than the traditional two-stage approach, which is based on model fitting to individual data followed by statistical analysis of parametric results, because they employ statistical models that explicitly account for average values of model parameters in the population (fixed effects) and their random intra- and interindividual variability (random effects). The modeling approach also includes the description of between-animal variability of parameters in terms of covariates. This was accomplished on the basis of physiological plausibility of results, statistical criteria for precision of parameter estimates, and minimum model complexity (14); e.g., a covariate was included into the model if the corresponding multiplicative regression parameter was estimated with a significance level of at least $P = 0.05$, being different from zero. Statistical analyses were performed using the NLME package (14) within the statistical programming environment (15). The simulation

![Fig. 1. Insulin concentration profiles (means ± 2 SE) measured in various classes of animals grouped according to the injected glucagon-like peptide-1 (GLP-1) dose.](image-url)
model of equations 1 and 2 has been produced with the software tool PANSYM (18).

Parameter estimates are reported in original units by back transformation (exponentiation) of actually estimated values. Consequently, figures of precision are given in terms of coefficients of variation, calculated as 100 times the standard error of the log-transformed parameter, and of lower and upper limits of 95% confidence intervals (CI) calculated by back-transforming the corresponding 95% CI given as estimates ± 1.96 SE.

RESULTS

GLP-1 strongly amplified glucose-stimulated insulin secretion (Fig. 1). The dose-response relationship between the

Fig. 2. Box plots of total area under the insulin concentration curves (AUC; over 50 min) vs. injected GLP-1 doses. Except for the control group (dose = 0), spacing along the abscissa essentially reflects the logarithm of the dose. Boxes indicate interquartile range (25–75%) with line of median. Dashed lines extend to data points that are no more than 1.5 times the interquartile range from the box. ▼, Extreme data points.

Fig. 3. Average glucose concentration profiles with 95% confidence intervals (CIs) (means ± 2 SE; ● and whiskers) and average model predictions (solid lines) obtained in various classes of animals grouped according to the injected GLP-1 dose.
logarithm of GLP-1 dose and total insulin secretion, quantified through the area under the concentration curve (AUC) in 50 min, was nearly linear (Fig. 2). Compared with the control group, the geometric mean of insulin AUC was significantly higher at any GLP-1 dose level \(P < 0.01\) for dosage groups 0.03 (nmol/kg) and 0.1, \(P < 0.001\) for groups 0.3 (nmol/kg) and 1.0, and \(P < 0.0001\) for the remaining groups.

A model-independent analysis of glucose profiles showed that the glucose disappearance constant, \(K_G\), calculated in the various GLP-1 dosage groups as the logarithmic slope of average glucose concentrations \(\leq\) 20 min, increased with administered GLP-1 dose up to 200%. The accelerated normalization of glucose in the higher GLP-1 dose groups was accompanied by a mild hypoglycemia and a slower return to basal toward the end of the experiments.

The minimal model combined with the population NLME approach described experimental data as shown in Fig. 3, which depicts the average glucose concentration profiles and model predictions obtained in the various groups of animals with different doses of injected GLP-1.

The final optimal population model selected according to statistical and heuristic criteria consisted of fixed effects associated with the four standard minimal model parameters (\(S_G\), \(S_t\), \(V_G\), and \(P_2\)) and an effect on the insulin action parameter \(P_2\) associated with the injected GLP-1 dose (Table 1). This latter effect reached the statistical significance level of \(P < 0.05\) only for GLP-1 doses of 10.4 (nmol/kg) \((P = 0.005)\) and 31.2 \((P = 0.010)\) and only a borderline value \((P = 0.07)\) for the dose of 3.0 (nmol/kg). With regard to the random effects, i.e., the unexplained between-animal variations of kinetic parameters, their estimated standard deviations corresponded to random unexplained between-animal variations of kinetic parameters, 3.0 (nmol/kg). With regard to the random effects, i.e., the unexplained between-animal variations of kinetic parameters, their estimated standard deviations corresponded to random unexplained between-animal variations of kinetic parameters, 3.0 (nmol/kg). With regard to the random effects, i.e., the unexplained between-animal variations of kinetic parameters, their estimated standard deviations corresponded to random unexplained between-animal variations of kinetic parameters, 3.0 (nmol/kg). With regard to the random effects, i.e., the unexplained between-animal variations of kinetic parameters, their estimated standard deviations corresponded to random unexplained between-animal variations of kinetic parameters, 3.0 (nmol/kg).

The dependence of insulin action turnover rate, \(P_2\), on the GLP-1 dose is represented in Fig. 4, showing the point estimates of the coefficient, together with the 95% CI, that multiplies the reference, zero-dose value of \(P_2\) at various dosage levels. Also, in this case, the average dependence of \(P_2\) on GLP-1 appears to be linearly related to the logarithm of the administered dose, except for the highest dose of 100 nmol/kg, whose 95% CI is nevertheless compatible with the trend observed at lower doses.

The implication of these changes in \(P_2\) on the minimal model of glucose disappearance is summarized graphically in Fig. 5, which shows the insulin action profiles, percent normalized with respect to \(S_G\), calculated with the actual \(P_2\) values for each GLP-1 dosage level (solid line) and with the \(P_2\) value at zero GLP-1 dose (dashed line). The interpretation of Fig. 5 is that GLP-1 increases insulin action not only through augmented insulin secretion but also with an increase of \(P_2\), which amplifies insulin-dependent vs. insulin-independent glucose disposal at higher glucose concentration values.

Table 1. NLME minimal model parameter estimates (fixed effects) and figures of precision

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>%CV</th>
<th>Lower 95%</th>
<th>Upper 95%</th>
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<tbody>
<tr>
<td>(S_G)</td>
<td>0.0628</td>
<td>3.7</td>
<td>0.0584</td>
<td>0.0676</td>
</tr>
<tr>
<td>(S_t)</td>
<td>3.024 \times 10^{-5}</td>
<td>7.1</td>
<td>2.632 \times 10^{-5}</td>
<td>3.475 \times 10^{-5}</td>
</tr>
<tr>
<td>(V_G)</td>
<td>0.00711</td>
<td>1.5</td>
<td>0.00691</td>
<td>0.00732</td>
</tr>
<tr>
<td>(P_2)</td>
<td>0.0207</td>
<td>24.3</td>
<td>0.0129</td>
<td>0.0334</td>
</tr>
<tr>
<td>(P_2) (0.03)*</td>
<td>0.69†</td>
<td>1.23</td>
<td>51.5</td>
<td>0.45</td>
</tr>
<tr>
<td>(P_2) (0.1)</td>
<td>0.46</td>
<td>1.42</td>
<td>46.9</td>
<td>0.57</td>
</tr>
<tr>
<td>(P_2) (0.3)</td>
<td>0.49</td>
<td>1.26</td>
<td>33.5</td>
<td>0.66</td>
</tr>
<tr>
<td>(P_2) (1.0)</td>
<td>0.27</td>
<td>1.50</td>
<td>36.3</td>
<td>0.73</td>
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<tr>
<td>(P_2) (3.0)</td>
<td>0.07</td>
<td>1.82</td>
<td>32.7</td>
<td>0.96</td>
</tr>
<tr>
<td>(P_2) (10.4)</td>
<td>0.005</td>
<td>1.94</td>
<td>23.2</td>
<td>1.23</td>
</tr>
<tr>
<td>(P_2) (31.2)</td>
<td>0.01</td>
<td>2.42</td>
<td>34.0</td>
<td>1.24</td>
</tr>
<tr>
<td>(P_2) (100)</td>
<td>0.23</td>
<td>1.41</td>
<td>29.2</td>
<td>0.80</td>
</tr>
</tbody>
</table>

NLME, nonlinear mixed effects; CV, coefficient variation; \(S_G\), glucose effectiveness; \(S_t\), insulin sensitivity index, \(V_G\), distribution volume; \(P_2\), turnover rate of insulin action. *Multiplicative coefficients for \(P_2\) at the indicated glucagon-like peptide-1 (GLP-1) dose in parentheses, e.g., at a dose of 0.03 (nmol/kg), \(P_2 = 0.0207 \times 1.23 = 0.0255\), i.e., +23%; †P values represent significance levels of the corresponding log-transformed parameter being different from zero, and thus of the multiplicative factor being different from 1, corresponding to the null hypothesis of no effect of GLP-1 on \(P_2\).
DISCUSSION

The present results provide an interpretation of the between-treatment differences observed in glucose disappearance under a wide range of GLP-1 doses. The main result is that the turnover rate constant for remote insulin action, $P_2$, is significantly and importantly increased by GLP-1 administration. This means that insulin action $\left[ X(t) \right]$ in the minimal model (equation 2) follows more rapidly suprabasal variations in insulin concentration $I(t)$. From a physiological perspective, this can be interpreted as an acceleration of the onset of insulin action on glucose disappearance at the beginning of the test or a more rapid return to basal at the end. However, although the total area under the insulin action curve, extrapolated to infinity, is independent on $P_2$, an earlier increase in insulin action when glucose is still high enhances insulin-dependent glucose disposal given by the product of insulin action and glucose concentration.

A possible explanation of faster dynamics of insulin action may be related to the "minimal model" interpretation of $S_1$, defined as the ability of insulin to enhance glucose disappearance and inhibit glucose production (5). Since GLP-1 suppresses glucagon secretion by the $\alpha$-cells of the pancreas (16), one possibility would therefore be that increasing doses of GLP-1 may accelerate suppression of hepatic glucose production (HGP) during IVGTT without affecting the lowest value of HGP reached during the test. This would be quantified by the minimal model in terms of increased $P_2$ and unaltered $S_1$. This may be achieved by augmented suppression of glucagon secretion by GLP-1, which is a possibility that needs to be explored in more detail.

Another possible interpretation for an increase in the remote insulin turnover parameter $P_2$ can be derived from the experimental observation that the time profile of insulin action, $X(t)$, mimics the insulin concentration profile in lymph (20). An increase of parameter $P_2$ can therefore also be interpreted in terms of a more rapid equilibration of insulin concentration between plasma and interstitial fluids. This can, in principle, be explained by hypothesizing a short-term effect of GLP-1 on tissue perfusion. In fact, endothelial-dependent vasorelaxant effects of GLP-1 have been observed experimentally in rats with regard to pulmonary circulation both in dissected pulmonary artery rings and with respect to vascular tone of perfused lungs (8). Similar vasorelaxant activity, but not related to endothelial function linked to nitric oxide, has also recently been observed in conduit (femoral) arteries (10). These observations indicate the presence of GLP-1 receptors in the vascular system in addition to specific organs (19), which suggests that acute administration of GPL-1 may also exert a direct effect on peripheral microvessels ameliorating tissue perfusion and improving diffusion between plasma and interstitial fluids of medium-sized molecules such as insulin.

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**Fig. 5.** Reconstructed insulin action (equation 2), % normalized with respect to glucose effectiveness ($S_0$), using the average insulin profiles shown in Fig. 1 for each GLP-1 dosage level. Solid lines correspond to the characteristic $P_2$ value of the corresponding dosage level; dashed lines correspond all to the same $P_2$ value estimated in the group with zero GLP-1 dose.
Since the minimal model analysis is based on plasma insulin concentration data, an increased $P_2$ does not likely depend (apart from possible modeling artifacts described below) upon insulin concentration levels and thus neither on insulin secretion nor on insulin clearance. With regard to this latter insulin clearance, elimination and signaling of insulin might have common pathways, but from the minimal model perspective they are completely independent.

When giving a physiological interpretation to these results, one should consider a prolonged below-basal glucose concentration (even if not a marked hypoglycemia) as an unusual aspect of glucose regulation. In particular, counterregulatory mechanisms strongly influence glucose kinetics and may alter results and interpretations based on minimal model analysis. This statement is supported by recent results from the insulin-modified IVGTT in normal tolerant subjects, in whom the test was repeated with additional glucose infusion to clamp glucose above 5.5 mmol/l and avoid temporary hypoglycemia and the excitation of counterregulatory mechanisms (6). The main result was that, with the glucose clamp experiments, the estimates of $S_1$ were increased and those of $P_2$ decreased. This shows that in normal glucose-tolerant subjects both $S_1$ and the duration of insulin action may be underestimated with insulin-modified IVGTT because of counterregulatory mechanisms. This could explain the increase of $P_2$ with GLP-1, because it has been observed that GLP-1 does not impair counterregulation to hypoglycemia (9). Nevertheless, the present analysis did not evidence any reduction with GLP-1 in $S_1$, which should have been observed in presence of counterregulatory response.

Results presented in this study lead to a different interpretation drawn in a previous study (3) regarding the effect of GLP-1 on insulin action and glucose disappearance. The new results suggest that GLP-1 accelerates with a nonlinear dose response the action of insulin in stimulating glucose disappearance rather than depressing $S_1$, as suggested by Ahrén and Pacini (3). The statistical approach used for identifying the minimal model parameters and their dependence on GLP-1 dose in a large group of animals adopted here makes an efficient use of experimental information, allowing the analysis of all data simultaneously and evaluating more accurately competing hypotheses. In this regard, the NLME procedure was not able to disentangle, through random effects, between-animal variability of $S_1$ and $P_2$. This means that experimental data did not provide sufficient information on the separate processes of time lag between variations in plasma insulin and variations in insulin action, characterized by $P_2$, and the actual strength of insulin action on glucose disappearance quantified by $S_1$. Nevertheless, a random effect associated with $P_2$ was found to be more effective in improving minimal model-based data predictions than between-animal variability of $S_1$.

A differentiation between pharmacological effects of GLP-1 on static vs. dynamic characteristics of insulin action (expressed through $S_1$ and $P_2$, respectively) is relevant in view of their joint contribution to short-term glucose regulation (13). These two aspects of insulin action cannot, however, be assessed independently. Although $S_1$ can in principle be measured in steady-state clamp experiments, the quantification of the time lag between changes in plasma insulin and changes in glucose disappearance rate requires necessarily dynamic experimental conditions. In this regard, the minimal model helps to disentangle both insulin-independent from insulin-dependent glucose disappearance and $S_1$ from insulin action kinetics.

The accelerating effect on insulin action emerges from this analysis as the most plausible direct interaction of GLP-1 with glucose kinetics. Two different plausible mechanisms explaining such an effect coexist, viz., inhibition of HGP and vasorelaxant effect on peripheral vessels. Hence, in addition to stimulating insulin secretion and reducing the fractional insulin clearance (4), GLP-1 also accelerates the insulin action following a glucose challenge. Therefore, at least three insulin-dependent mechanisms contribute to the glucose-lowering action of GLP-1, to which the inhibition of glucagon secretion is added. The novel effect of GLP-1 to accelerate insulin action further corroborates the viewpoint that GLP-1 represents an important coregulatory factor in normal physiology of glucose homeostasis and supports novel therapy based on this incretin.

ACKNOWLEDGMENTS

We are grateful to Lena Kvist and Lilian Bengtsson for expert technical assistance.

GRANTS

This study was supported in part by grants from the Swedish Research Council (6834), the Swedish Diabetes Association, the Pähltsson Foundation, Region Skåne, Faculty of Medicine, Lund University, and by funds from Regione Veneto (Progetto Biotech).

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