Intestinal transit of a glucose bolus and incretin kinetics: a mathematical model with application to the oral glucose tolerance test

Serenella Salinari,1 Alessandro Bertuzzi,2 and Geltrude Mingrone3

1Department of Computer and System Science, University of Rome “Sapienza”; 2Institute of Systems Analysis and Computer Science, National Research Council; 3Department of Internal Medicine, Catholic University, School of Medicine, Rome, Italy

Submitted 30 July 2010; accepted in final form 28 February 2011

Salinari S, Bertuzzi A, Mingrone G. Intestinal transit of a glucose bolus and incretin kinetics: a mathematical model with application to the oral glucose tolerance test. Am J Physiol Endocrinol Metab 300: E955–E965, 2011. First published March 1, 2011; doi:10.1152/ajpendo.00451.2010.—The rate of appearance (Ra) of exogenous glucose in plasma after glucose ingestion is presently measured by tracer techniques that cannot be used in standard clinical testing such as the oral glucose tolerance test (OGTT). We propose a mathematical model that represents in a simple way the gastric emptying, the transport of glucose along the intestinal tract, and its absorption from gut lumen into portal blood. The model gives the Ra time course in terms of parameters with a physiological counterpart and provides an expression for the release of incretin hormones as related to glucose transit into gut lumen. Glucose absorption was represented by assuming two components related to a proximal and a distal transporter. Model performance was evaluated by numerical simulations. The model was then validated by fitting OGTT glucose and GLP-1 data in healthy controls and type 2 diabetic patients, and useful information was obtained for the rate of gastric emptying, the rate of glucose absorption, the Ra profile, the insulin sensitivity, and the glucose effectiveness. Model-derived estimates of insulin sensitivity were well correlated (r = 0.929 in controls and 0.886 in diabetic patients) to data obtained from the euglycemic hyperinsulinemic clamp. Although the proposed OGTT analysis requires the measurement of an additional hormone concentration (GLP-1), it appears to be a reasonable choice since it avoids complex and expensive techniques, such as isotopes for glucose Ra measurement and direct assessment of gastric emptying and intestinal transit, and gives additional correlated information, thus largely compensating for the extra expense.

THE MATHEMATICAL MODEL ANALYSIS of an intravenous glucose tolerance test (IVGTT) is facilitated by the fact the amount of glucose injected is known. In contrast, an oral glucose tolerance test (OGTT) is characterized by uncertainty both in the amount of glucose absorbed and in its absorption rate, which results in the time course of exogenous glucose delivery to plasma. The IVGTT models (3, 7, 31) represent the glucose delivery as a bolus dose of known amount, D, that abruptly raises plasma glucose concentration of an amount D/VG, with VG being the glucose distribution volume. In the OGTT, by contrast, the time course of the rate of appearance (Ra) of exogenous glucose in plasma cannot be specified a priori. Given the ingested dose, indeed, several factors contribute toward affecting the Ra: the rate of gastric emptying of ingested glucose, the extent of intestinal absorption during the intestinal transit, and the hepatic uptake of portal glucose. Because of these uncertainties, the Ra time course in the OGTT and the meal test has been represented by a piecewise linear function, with the time of break points assigned and values that are estimated from the data (8, 9, 10).

Experimental determinations of the Ra of ingested glucose in plasma have been obtained using tracer techniques. Ferrannini and colleagues (19, 20) determined the Ra of ingested glucose in healthy controls and in type 2 diabetic patients. The Ra showed a comparable time course in the two groups, reaching a peak of 6–6.5 mg·min⁻¹·kg⁻¹ ≈ 30 min after glucose ingestion (1 g/kg body wt), with 73–76% of the oral load recovered in the circulation in the 210-min study period. Ra data during the OGTT in type 2 diabetic and in control subjects were reported by Thorburn et al. (37). Other studies where the Ra of oral glucose was measured in the OGTT or following a mixed meal are also available (see, for instance, Refs. 4, 9, 10, 40, and 41). Overall, most of the experimental data show a common pattern of Ra, where an initial peak is followed by a more or less smoothed second peak that slowly declines toward zero. The rate of gastric emptying and the small intestine transit time appear to be the main factors in determining the glucose Ra profile. Schirra et al. (35) measured the gastric retention after glucose ingestion in humans and fitted the data by a power exponential function, as proposed by Elashoff et al. (17). A retention of 5% of the glucose load was estimated to occur at about 130 min and at ~193 min for a load of 50 and 100 g, respectively. Different mathematical models describing the gastric emptying and the intestinal absorption have been proposed over time (5, 11, 26, 36). Although limited data have been found on the small intestinal transit time of glucose, this time appears to be in the range of 3–4 h (1, 39).

In the present study we propose a mathematical model that explicitly represents, in the simplest possible way, the gastric emptying, the transport of glucose along the intestinal tract, and its absorption from the gut lumen into the portal blood. The model may be useful to 1) express the time course of the rate of exogenous glucose appearance (Ra) in terms of parameters having a physiological counterpart, 2) suggest a possible model for the release of incretin hormones by enteroendocrine cells as related to the transit of glucose into the gut lumen, since intraluminal glucose appears to be a triggering factor for the secretion of these hormones (2, 12, 23), and 3) provide a physiological approach to the analysis of OGTT data by a mathematical model, also giving an estimate of the insulin sensitivity. An analysis of OGTT data from healthy controls and type 2 diabetic (T2D) patients, using the glucagon-like peptide-1 (GLP-1) concentration data to help estimate the Ra time course, is presented.
RESEARCH DESIGN AND METHODS

Subjects

Six (4 women and 2 men) morbidly obese [body mass index (BMI) = 53.4 ± 8.1 kg/m², age 41 ± 9 yr (means ± SD)] T2D patients and eight normotolerant [according to the American Diabetes Association criteria (17a)] sex- and age-matched volunteers (4 women and 4 men, BMI = 24.7 ± 0.6 kg/m², age 45 ± 6 yr) were studied. Glycosylated hemoglobin (Hb A1c) ranged from 7.5 to 9.5%.

Study Protocol

At the time of the study, all subjects were on a diet with the following average composition: 60% carbohydrate, 30% fat, and 10% protein (1 g/kg body wt). This dietary regimen was maintained for 1 wk before the study. In all of the patients and healthy controls, an OGTT and a euglycemic hyperinsulinemic clamp were performed randomly.

The study protocol was approved by the Institutional Ethics Committee of the Catholic University of Rome. The nature and purpose of the study were carefully explained to all subjects before they provided their written consent to participate.

Body Composition

On a separate day, total body water (TBW) was determined using 0.19 MBq H2O in 5 ml of saline administered as an intravenous bolus injection. Blood samples were drawn before and 3 h after the injection. Radioactivity was determined in duplicate on 0.5 ml of plasma in a β-scintillation counter (Model 1600TR; Canberra-Packard, Meriden, CT). Corrections were made for nonaqueous hydrogen exchange. Water density at body temperature was assumed to be 0.99371 kg/l. TBW (kg) was computed as

\[ \text{TBW (kg)} = \frac{0.5 \times 0.99371}{\text{dilution space (liters)}} \]

Fat-free mass was obtained as TBW/0.732 (34) and fat mass as the difference between body weight and fat-free mass.

OGTT

After an overnight fast, a standard 75-g OGTT was performed, with blood sampling at 0, 30, 60, 90, 120, 150, and 180 min. Samples were placed in chilled tubes, and plasma was separated within 20 min and stored at −70°C.

Euglycemic Hyperinsulinemic Clamp

Peripheral insulin sensitivity was evaluated by the euglycemic hyperinsulinemic clamp (13). The subjects were fasted overnight. Briefly, after one cannula was inserted in a dorsal hand vein for sampling arterialized venous blood and another in the antecubital fossa of the contralateral arm for infusions, the subjects rested in the supine position for at least 1 h. They were then warmed in a heated air box set at 60°C to obtain arterialized blood samples. Insulin sensitivity was determined during a primed constant insulin infusion at the rate of 6 pmol·min⁻¹·kg body wt⁻¹. In diabetic subjects, rapid insulin and 20 meq potassium phosphate in saline were infused overnight to maintain the glycemia in a normal range. The plasma glucose concentration was clamped at the fasting value for 2 h throughout the insulin infusion by means of a variable glucose infusion adjusted on the basis of blood glucose determinations every 5 min. Insulin sensitivity was determined during the last 40 min of the clamp by computing the whole body glucose uptake (M, μmol·min⁻¹·kg body wt⁻¹) during steady-state euglycemic hyperinsulinemia.

Analytical Procedures

Blood samples were drawn into EDTA-evacuated tubes, with the addition of 30 μl (10 μl/ml blood) of dipeptidyl peptidase IV inhibitor (LC0014; Linco Research, St. Charles, MO). The plasma was immediately separated by centrifugation at 4°C and stored at −80°C until assay. These samples were not thawed until hormone assays were performed.

Plasma glucose was measured by the glucose oxidase technique on a Beckman Glucose Analyzer (Beckman, Fullerton, CA). Plasma insulin was assayed by microparticle enzyme immunoassay (Abbott, Pasadena, CA) with sensitivity of 1 μU/ml and intra-assay coefficient of variation of 6.6%.

Plasma total GLP-1 was measured with commercially available RIA [GLP (total) RIA kit; Linco Research]. The sensitivity limit was 3 pM, and the intra- and interassay coefficients of variation were <20%.

Immunoreactive glucose-dependent insulinoptive polypeptide (GIP) levels were determined using 0.1 ml of plasma in a human GIP RIA kit (Peninsula Laboratories, Belmont, CA). Intra-assay variation was 8% and interassay variation was 8% for 20 and 80 pmol/l standards, respectively.

Mathematical Model

Gastric delivery and transit of glucose bolus into the gut lumen. The progression of the glucose bolus through the intestinal lumen is represented as a one-dimensional process in which all glucose particles are transported from the proximal to the distal end of the small intestine with a constant speed, u. A second essential assumption, to be motivated in the following, is that glucose absorption by the enterocytes obeys a first-order linear kinetics with a rate coefficient, γ, that may depend on the spatial position along the intestine because of a nonuniform regional distribution of glucose transporters (and possibly on other variables).

We associate with the small intestine a longitudinal coordinate, z, that measures the distance from the pylorus (z = 0). The amount of intraluminal glucose per unit length is denoted as q(z, t) so that q(z, t)Δz is the glucose amount contained at time t in the intestinal segment from z to z + Δz. A mass conservation equation for q (indicated in the following as luminal glucose density and expressed, e.g., in mmol/cm) may be written as follows:

\[ \frac{\partial q}{\partial t} + u \frac{\partial q}{\partial z} = -\gamma q, \quad z \geq 0, t \geq 0, \] (1)

with γ = γ(z). Equation 1 is the simplest model of convective one-dimensional transport; a derivation of this equation may be found in APPENDIX A: TRANSPORT EQUATION. Since no glucose is assumed to be present in the gut at the initial time, u = 0, the initial condition for Eq. 1 is q(z, 0) = 0.

The glucose bolus is delivered to the small intestine through gastric emptying. If h(t) is the fraction of glucose retained in the stomach at time t after glucose ingestion, the glucose amount retained in the stomach after a dose, D, is Dh(t). The rate of glucose delivery to duodenum [denoted here as η(t) and expressed, e.g., as mmol/min] is

\[ \eta(t) = -D \times \text{dh/dt}. \]

Although the delivery is completed in a finite time interval, gastric retention has been represented by a power exponential function, h(t) = exp(-κt)^p (17, 35), so that the rate of glucose delivery to duodenum is given by η(t) = Dβ \exp(-κt)^p. Based on the analysis of experimental Rg data, Dalla Man et al. (11) proposed a more complex expression for the gastric delivery rate by assuming that the rate coefficient of gastric emptying is a function of the gastric content of glucose.

The delivery of glucose to duodenum is represented here by prescribing to Eq. 1 the following condition at the boundary z = 0 (influx at the boundary equal to gastric delivery rate):

\[ q(0, t) = \begin{cases} (1/u) \eta(t), & 0 \leq t \leq 0 \\ 0, & t \geq 0 \end{cases} \] (2)

with 0 the time required for the gastric emptying of the glucose dose into the gut. We will denote q(0, t) as q0(t).
The solution of Eqs. 1 and 2 is found by the method of characteristics (42) and is given by

\[ q(z, t) = \begin{cases} 
q_B(t - z/u) \exp \left[ -\frac{(1/u) \int_0^z c(\zeta, t) \, d\zeta} \right], & 0 \leq t - z/u \leq 0, \ z \geq 0 \\
0, & \text{elsewhere.} 
\end{cases} \]

Equation 3 simply states that the glucose found at \((z, t)\) is equal to the glucose that has entered the gut \((z = 0)\) at the previous time, \(t - z/u\), reduced by the glucose absorbed through gut wall during the transit from 0 to \(z\). As shown by the inequalities in Eq. 3, \(q(z, t)\) is nonzero only in the \(z\)-interval from max \([0, u(t - 0)]\) to \(u\). When \(h(t)\) vanishes for \(t \to \infty\), \(q\) will be nonzero for \(0 \leq z \leq u\).

If \(L\) is the length of the glucose-absorbing region, the “head” of the intraluminal glucose bolus (glucose particles entered the intestine at \(t = 0\)) starts leaving that region at a time equal to \(L/u\). \(L/u\) is thus the glucose transit time in the absorbing region of the small intestine. We note that \(q\) may be virtually zero at \(z = L\), which means that almost all ingested glucose undergoes absorption during the intestinal transit.

\(R_a\) of glucose in plasma. The glucose taken up from gut lumen is internalized in the enterocytes by apically located transporters (the sodium-dependent glucose cotransporter SGLT1 and possibly the fructose transporter GLUT5) (16). Glucose is then released from the cells into the interstitial space via GLUT2 and enters the blood stream. It has also been found that luminal glucose may transiently recruit GLUT2 to the brush-border membrane (25, 38). SGLT1, GLUT2, and GLUT5 gene expression was found to be nonuniformly distributed along the intestinal mucosa, and we define two space-dependent absorption rate coefficients, \(\gamma_1(z)\) and \(\gamma_2(z)\), with the total transport rate being \(p(z, t) = [\gamma_1(z) + \gamma_2(z)] q(z, t) = \gamma(z) q(z, t)\). This term appears as a loss term, i.e., \(-\gamma(z) q(z, t)\), in the right-hand side of Eq. 1.

The integral of \(p(z, t)\) over the length \(L\) of the absorbing region gives the total glucose absorbed at the time \(t\). Not all of the glucose absorbed is released into the portal blood because of glucose metabolism in the enterocytes. Moreover, portal glucose undergoes hepatic uptake before entering the general circulation. Denoting by \(1 - f\) the fraction (taken constant) of glucose lost in the transport from gut lumen to plasma, the \(R_a\) takes the expression

\[ R_a(t) = \int_0^L \gamma(z) q(z, t) \, dz, \quad t \geq 0, \]

with \(q(z, t)\) given by Eq. 3. Because \(q\) will be nonzero only in a portion of gut lumen, the integral in Eq. 5 may be carried out in the \(z\)-interval from 0 to \(\min[u(L), L]/u\) for \(t < 0\) and from \(u(t - 0)\) to \(\min[u(L), L]\) for \(t \geq 0\). In the absence of data on the regional distribution of the transporters, \(\gamma_1(z)\) and \(\gamma_2(z)\) may be represented as piecewise constant functions or proportional to Gaussian functions.

A closed-form expression of the \(R_a\) time course is easily obtained under two simplifying assumptions: the rate of glucose delivery to duodenum is exponential, and the rate coefficient \(\gamma\) of glucose absorption is constant (see APPENDIX B: EXponential DELivery and UNIFORM ABSorption).

Kinetics of GIP and GLP-1. The secretion of incretin hormones by the enteroeccrine cells occurs in response to the transit of nutrients in the intestinal lumen (2, 12, 23). \(K\) cells that secrete the GIP are found primarily in duodenum and upper jejunum, but GIP-producing cells may be found in the mucosa of the entire small intestine. GLP-1-secreting \(L\) cells are found with the highest density in distal ileum but also in the rest of the small intestine and in the large intestine, too. A population of duodenal cells in which GIP and GLP-1 are colocalized has also been demonstrated (29).

The rate of absorption, rather than the simple presence of nutrients in the gut, determines GIP release (2, 12). GLP-1 plasma concentration displays a biphasic pattern, with an early phase followed by a longer second phase (2). GLP-1 secretion appears to correlate with gastric emptying provided that the glucose delivery rate exceeds a threshold (35). Because the majority of \(L\) cells are found in the lower regions of the small intestine, the occurrence of the first phase has been explained by the existence of a neural or endocrine duodenal-ileal loop (2, 12). However, the direct contact of nutrients with the more proximally located \(L\) cells could explain the rapid initial increase in plasma GLP-1.

Another major point for describing the kinetics of the hormones is the mechanism of removal of GIP and GLP-1. It has been found that the plasma half-life of exogenously administered incretins is 1–2 min for GLP-1 and 7 min for GIP (2, 12). Enzymatic degradation by the dipeptidyl peptidase IV occurs mainly in the liver and also in vessels close to \(L\) cells (2, 12).

We write the equation describing the kinetics of total GIP in plasma in the following form; see also (5)

\[ \frac{d}{dt} \text{GIP} = a_{\text{GIP}} (\text{GIP} - \text{GIP}_b) + b_{\text{GIP}} \text{GIP}_K, \quad \text{GIP}(0) = \text{GIP}_b, \]

where \(\text{GIP}\) denotes the GIP plasma concentration (basal concentration, \(\text{GIP}_b\)) and \(a_{\text{GIP}}\) the degradation rate constant. The term \(b_{\text{GIP}}\) is the ratio between rate of GIP release and GIP distribution volume, and the release rate is taken proportional to the rate of glucose absorption by the \(K\) cells, \(\text{GIP}_K\). The absorption rate coefficient, \(\gamma_1\) will be nonzero only in the region where the \(K\) cells are present, and the coefficient
b_{\text{CAP}} will account for the “efficiency” of the mechanism of GIP secretion.

A similar equation can be written for the kinetics of total GLP-1:

\[
\frac{d}{dt} \text{GLP} = \alpha_{\text{GLP}}(-\text{GLP} + \text{GLP}_b) + b_{\text{GLP}}\varphi_L, \quad \text{GLP}(0) = \text{GLP}_b, \quad (7)
\]

with

\[
\varphi_L(t) = \int_{0}^{\min(L, L_1)} \gamma_L(z)q(z, t)dz,
\]

where GLP is the plasma concentration of GLP-1 (basal concentration, GLP_0). Because the release of GLP-1 occurs both in the upper small intestine and at distal ileal sites, we assume that the absorption coefficient \( \gamma_L(z) \) has a proximal and a distal component. Accordingly, the GLP-1 secretion will display an early phase, with profile similar to the rate of gastric delivery of glucose, and a second phase related to the transit of luminal glucose in the ileum. The constant parameters \( \alpha_{\text{GLP}} \) and \( b_{\text{GLP}} \) have meanings similar to those in the equation for GIP. A simplified analysis of GLP-1 and GIP kinetics in the intraduodenal glucose infusion experiments is reported in APPENDIX B: EXPONENTIAL DELIVERY AND UNIFORM ABSORPTION.

Two important points must be noted. It has been suggested that the direct contact of luminal glucose with L cells, rather than its absorption, stimulates GLP-1 release (2, 6, 12, 28, 35). If we assume that GLP-1 secretion is related to glucose concentration instead of absorption, the rate \( \varphi_L \) in Eq. 7 can be computed by setting \( \gamma_L(z) = 1 \), so the integral that gives \( \varphi_L \) will depend on the luminal glucose content. A second point is that the models of Eqs. 6 and 7 impose that incretin concentrations cannot be smaller than the basal values, in contrast with the observations. A more refined model might, for instance, account for the regulation of GLP-1 secretion by insulin (27).

As seen in the following, the rate coefficients of incretin removal from plasma, \( a_{\text{CAP}} \) and \( a_{\text{GLP}} \), are larger (especially for GLP-1) than those of gastric delivery and intestinal absorption. Thus, the incretin plasma concentration will be substantially dependent on the \( R_a \) profile. In fact, the GIP and GLP-1 concentrations were found to be correlated with the \( R_a \) (30, 40).

If the kinetics of GIP and GLP-1 is appropriately related to the intestinal transit of the glucose bolus and to the glucose absorption, the data of hormone concentrations in plasma might help in estimating the unknown parameters of the plasma \( R_a \) of the exogenous glucose, provided that direct measurements of the \( R_a \) are not usually available in a clinical setting.

Parameter Estimation

The model parameters were estimated by minimization of a weighted least-square index using a constrained Levenberg-Marquardt minimization routine of the MATLAB library. The coefficients of variation of the estimates were found to be <20%.

Statistics

All of the data were expressed as means \( \pm \) SD unless otherwise specified. The Kolmogorov-Smirnov test for intergroup comparisons was used. Two-sided \( P < 0.05 \) was considered significant. Nonparametric Spearman’s correlation (SPSS for Windows version 10) was used to assess the linear relationships between single variables.

RESULTS

Model of \( R_a \) With Exponential Delivery and Uniform Absorption

Two illustrative examples of the time course of the \( R_a \) predicted under the assumption of exponential delivery and uniform absorption (APPENDIX B: EXPONENTIAL DELIVERY AND UNIFORM ABSORPTION) by Eqs. B2a and B2b, are shown in Fig. 1, A and C. The corresponding rate of gastric delivery, which is maximal at \( t = 0 \), is also plotted. The parameters of the simulation were chosen to give an intestinal transit time (L/U) equal to 150 min, and the values of the rate coefficient of gastric delivery, \( k \), and of the rate coefficient of intestinal absorption, \( \gamma \), were interchanged in Fig. 1, A and C. An abrupt change in the slope of the \( R_a \) profile at \( t = L/U \) is clearly visible only in Fig. 1C. Using Eqs. B2a and B2b, the decrease in slope is found equal to \( fDk \times \exp(-\gamma L/U) \), so in this example the slope decrease is evident when the time constant of absorption, \( 1/\gamma \), is large enough compared with L/U. As expected, if \( k \) is substantially smaller than \( \gamma \) (Fig. 1A), the \( R_a \) is governed by the gastric delivery.

The profiles of the luminal glucose density along the small intestine at three time values are depicted in Fig. 1, B and D. The profiles show the progression of the glucose bolus through the intestine, with the bolus head moving as \( z = ut \). As seen by Eq. B1, the glucose density \( q \), as a function of \( z \), has a decreasing or an increasing exponential pattern according to the positive or negative sign of the difference \( \gamma - k \). We observe that the initial part of the \( R_a \) time course, given by Eq. B2a, does not change if the values of \( k \) and \( \gamma \) are interchanged. Moreover, for large values of \( \gamma × L/U \), the \( R_a \) predicted by the model is almost insensitive to the ratio \( L/U \) (see Fig. 1A). This feature of the model might preclude a reasonable estimation of the parameters from the experimental data, especially if these are collected for a short time interval (e.g., 120 min). However, the percent of the glucose dose that escapes absorption, computed from the \( R_a \) area under the curve (AUC) from 0 to infinity, equals 0.25% of DI with the parameters of Fig. 1A \( k < \gamma \) and 9.07% with parameters of Fig. 1C \( k > \gamma \). So the glucose amount entering the circulation (the fraction \( f \) being equal) will differ in the two cases.

Model of \( R_a \) With Nonuniform Intestinal Absorption

The profile of the \( R_a \) predicted by the model with nonuniform intestinal absorption may take quite different forms. Here, we have hypothesized the presence of two glucose transporters, one located in the upper small intestine (duodenum-jejunum) and the other in the lower small intestine (jejunum-ileum). On an empirical basis, the corresponding absorption rate coefficients, \( \gamma_L(z) \) and \( \gamma_G(z) \) respectively, were taken to be proportional to Gaussian functions with means \( z_1 \) and \( z_2 \), standard deviations \( \sigma_1 \) and \( \sigma_2 \), and proportionality coefficients \( c_1 \) and \( c_2 \). The profiles chosen for the proximal and the distal component, \( \gamma_L(z) \) and \( \gamma_G(z) \), and of \( \gamma(z) \) are depicted in Fig. 2A. The proximal transporter is located in the duodenum and proximal jejunum (\( z_1 = 0 \) and \( 3\sigma_1 = 105 \) cm), whereas the distal transporter is present in the whole small intestine with maximal density in the midileum. Figure 2B shows the rate of gastric delivery, \( \eta(t) \), computed by assuming the power exponential function for the gastric retention of glucose \( \eta(t) \) maximal at 12 min in this simulation], and the \( R_a \). Contrary to the case of \( \gamma \) constant, when the absorption rate coefficient changes along the intestine, the \( R_a \) profile may present a “hump” in the descending branch, as was also observed in some experimental data (4, 9, 10, 11, 19, 20, 37, 41). Figure 2B also reports the time course of \( R_{a1} \) and \( R_{a2} \), which are the components of \( R_a \).
due to the glucose taken up by the proximal and the distal transporters, respectively. According to the model, the occurrence of the hump is related to the entrance of the glucose bolus in the region of the distal transporters. The profile of \( q(z,t) \) along the small intestine at four times is depicted in Fig. 2C.

Figure 2D shows different time courses of the rate of appearance, simulated by changing some of the model parameters. Compared with the \( R_a \) profile of Fig. 2B (solid line), the maximal \( R_a \) value is displaced to a later time if the coefficient \( c_1 \) is decreased and \( c_2 \) is increased (curve a). Still, with \( c_1 \) decreased and \( c_2 \) increased, if the distal transporter is more distally located, two well-separated peaks appear in the \( R_a \) (curve b). The rate constant \( k \) of gastric delivery was also increased in this simulation so that \( R_a \) declines more rapidly toward zero. By contrast, if \( k \) and the transit velocity \( u \) are decreased, the hump in the descending branch is no longer visible and the \( R_a \) response declines more slowly (curve c).

In the above simulations, the glucose amount that escapes absorption is between 0.96 and 1.85% of \( f_D \).

Simulation of Intraduodenal Glucose Infusion

In the intraduodenal glucose infusion experiments, glucose is infused at a constant rate in the duodenum and plasma glucose, and insulin and incretin concentrations are measured. For instance, infusion rates used were as follows: 1.1 or 2.2 kcal/min (1.53 or 3.06 mmol/min, respectively) over 180 min, with the catheter tip placed in the distal part of the descending duodenum (35); 2.33 mmol/min (normalized to 70 kg body wt) for 180 min, with catheter tip at \( 15 \) cm beyond the pylorus (22); 3.5 kcal/min (4.86 mmol/min) infused for 60 min into an isolated 60-cm segment of the proximal small intestine (short segment) or into the entire small intestine (long segment) (28); and 1 kcal/min (1.39 mmol/min) for 90 min in a duodenal or midjejunal location (6).

From the data in Schirra et al. (35), we observe that, when the infusion rate was doubled, the steady-state increment GIP was also doubled. This finding, confirmed by the results in Féry et al. (22), suggests that both the intestinal glucose absorption and the GIP secretion are essentially linear processes in the range of infusion rates considered so that the maximal duodenal absorption rate is not attained in these experiments.

To analyze the GLP-1 and GIP concentration data obtained in these experiments, the present model can be applied as shown in APPENDIX B: EXPONENTIAL DELIVERY AND UNIFORM ABSORPTION, assuming for simplicity that \( \gamma_l \), \( \gamma_L \), and \( \gamma_K \) are constant with \( z \). The ratio of the steady-state increment of the GLP-1 concentration over the glucose infusion rate, \( \Delta GLP/\eta \), provides an estimate of the quantity \( b_{GLP} \times \gamma_L/(a_{GLP} \times \gamma) \) according to Eq. B3. The estimation of the
quantity \( b_{GIP} \times \gamma_k(a_{GIP} \times \gamma) \) from the GIP concentration data is less straightforward, as shown by Eq. B4.

In Schirra et al. (35), the GLP-1 response was obtained only with the glucose infusion rate of 3.06 mmol/min, and the steady-state increment of active GLP-1 was \(-1.75\) pM, giving (active) \( \Delta GLP/\eta = 0.57 \times 10^{-9} \) min/l. The data in Little et al. (28), measured with a different experimental setup, show that no GLP-1 response was obtained with the short-segment infusion, whereas a response was elicited, but the steady state was not achieved in the 60-min observation period with the long-segment infusion. About 2 pM total GLP-1 was reported in (6) with the midjejunal glucose infusion of 1.39 mmol/min, so (total) \( \Delta GLP/\eta = 1.44 \times 10^{-9} \) min/l. These estimates may be compared with those obtained from the OGTT.

**Analysis of OGTT Data**

For the analysis of the OGTT, we represented the glucose kinetics and the insulin sensitivity as in the minimal model (9, 10). The minimal model equations were complemented with the \( \gamma_2(z) \) of GLP-1 kinetics. The GLP-1 profile, which reflects the progression of glucose load through the entire small intestine, may indeed be most informative on the distribution of glucose transporters. The equations used with the parameters involved are reported in \( \text{APPENDIX C: EQUATIONS FOR THE OGTT ANALYSIS.} \)

Because the mathematical model has several unknown parameters, some of these parameters were assigned a priori, as detailed in the following. For \( \gamma_1(z) \) and \( \gamma_2(z) \) in controls, in the absence of data on the regional distribution of intestinal glucose transporters in humans, we set \( z_1 = 0, \sigma_1 = 20 \) cm for the proximal transporter and \( z_2 = 350 \) cm, \( \sigma_2 = 120 \) cm for the distal transporter. Moreover, based on preliminary estimates, we assumed \( c_1 = c_2/2 \) with \( c = c_2 \) the parameter to be estimated from the data. For the T2D patients, we set \( z_1 = 50 \) cm, \( \sigma_1 = 70 \) cm, and \( c_1 = c_2/5 \).

Other parameters assigned were \( \beta = 1.1 \) (35), \( f = 0.87 \) (10), and \( a_{GIP} = (\ln 2)/1.5 \) min\(^{-1} \) (12). The glucose distribution volume, \( V_O \), was computed for each subject by the formula reported in Denti et al. (14) that accounts for sex, age, total body fat, and basal glucose concentration. Because the data were available \( \approx 180 \) min, and thus for a time likely shorter than the small intestine transit time (1, 39), we set \( L = 600 \) cm and \( u = 3 \) cm/min (\( L/u = 200 \) min). In the equation of GLP-1 kinetics we set \( \gamma_L = \gamma \), although \( \gamma \) cells are only a small fraction of the enterocytes; with this choice, \( b_{GIP} \) is actually expressed as rate of change of GLP-1 concentration (pM/min).
for unit glucose absorption rate by enterocytes (mmol/min). Because Eq. 7 imposes that GLP-1 concentration cannot be smaller than \( \text{GLP}_b \), in contrast with the data (see Fig. 3, B and D), we did not constrain \( \text{GLP}_b \) to equal the GLP-1 concentration at \( t/H_1 \) and left it as a parameter to be estimated.

In summary, the following parameters were estimated for each subject: \( k, c, S_G, S_I, p, \text{GLP}_b, \) and \( b_{\text{GLP}} \), where \( S_G \) is the glucose effectiveness, \( S_I \) the insulin sensitivity, and \( p \) the rate constant of the insulin action.

To estimate the model parameters, the insulin concentration was approximated as a piecewise linear function connecting the data points, and the glucose and GLP-1 time-concentration curves predicted by the model were fitted to the measured values. The estimation was performed by weighted least squares, with weights given by the squared inverses of the experimental data. Figure 3 shows the glucose and GLP-1 concentration data (Fig. 3, A and B, for controls, and Fig. 3, C and D, for T2D patients) and the model predictions computed with the average values of parameter estimates. The \( R_a \) profile, which is close to the GLP-1 concentration as expected, is plotted in Fig. 3, A and C.

Table 1. Individual (1–8) and average parameter estimates of the OGTT mathematical model for control subjects

<table>
<thead>
<tr>
<th>Parameter Estimate</th>
<th>( k \times 10^2 \text{ min}^{-1} )</th>
<th>( \Gamma \times 10^2 \text{ min}^{-1} )</th>
<th>( S_G \times 10^2 \text{ min}^{-1} )</th>
<th>( S_I \times 10^2 \text{ l}^{-1} \text{ min}^{-1} \text{ pM}^{-1} )</th>
<th>( p \times 10^2 \text{ min}^{-1} )</th>
<th>( b_{\text{GLP}} \times 10^4 \text{ l}^{-1} \text{ min}^{-1} )</th>
<th>( \text{GLP}_b \text{ pM} )</th>
<th>( S_I^\text{CL} \times 10^4 \text{ l}^{-1} \text{ min}^{-1} \text{ pM}^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.09</td>
<td>1.79</td>
<td>1.89</td>
<td>1.28</td>
<td>1.27</td>
<td>6.14</td>
<td>18.30</td>
<td>2.59</td>
</tr>
<tr>
<td>2</td>
<td>2.01</td>
<td>1.82</td>
<td>2.94</td>
<td>2.30</td>
<td>2.10</td>
<td>4.74</td>
<td>16.38</td>
<td>4.17</td>
</tr>
<tr>
<td>3</td>
<td>2.85</td>
<td>1.45</td>
<td>2.73</td>
<td>1.27</td>
<td>0.71</td>
<td>7.51</td>
<td>20.33</td>
<td>1.61</td>
</tr>
<tr>
<td>4</td>
<td>1.69</td>
<td>1.78</td>
<td>1.95</td>
<td>1.98</td>
<td>1.18</td>
<td>5.90</td>
<td>13.61</td>
<td>3.24</td>
</tr>
<tr>
<td>5</td>
<td>2.43</td>
<td>1.46</td>
<td>1.18</td>
<td>1.43</td>
<td>1.05</td>
<td>7.44</td>
<td>17.06</td>
<td>1.92</td>
</tr>
<tr>
<td>6</td>
<td>1.92</td>
<td>1.66</td>
<td>1.37</td>
<td>1.71</td>
<td>0.95</td>
<td>5.99</td>
<td>15.08</td>
<td>2.98</td>
</tr>
<tr>
<td>7</td>
<td>1.70</td>
<td>2.45</td>
<td>1.89</td>
<td>1.58</td>
<td>0.67</td>
<td>6.56</td>
<td>17.08</td>
<td>2.24</td>
</tr>
<tr>
<td>8</td>
<td>2.32</td>
<td>2.24</td>
<td>3.17</td>
<td>1.22</td>
<td>0.44</td>
<td>6.39</td>
<td>22.94</td>
<td>1.47</td>
</tr>
<tr>
<td>Average</td>
<td>2.13 ± 0.39</td>
<td>1.83 ± 0.35</td>
<td>2.01 ± 0.70</td>
<td>1.60 ± 0.38</td>
<td>1.05 ± 0.51</td>
<td>6.33 ± 0.89</td>
<td>17.60 ± 2.95</td>
<td>2.53 ± 0.91</td>
</tr>
</tbody>
</table>

Values for average parameter estimates are means ± SD. OGTT, oral glucose tolerance test; \( S_G \), glucose effectiveness; \( S_I \), insulin sensitivity; \( p \), rate constant of insulin action; GLP, glucagon-like peptide-1. \( S_I^\text{CL} \), insulin sensitivity values derived from the euglycemic hyperinsulinemic clamp.
population means ± SD. Instead of c, the tables report the \( z \)-averaged absorption rate coefficient, \( \Gamma \), computed as the integral of \( \gamma(z) \) divided by \( L \). The \( S_i \), expressed as \( \text{d}l\text{kg}^{-1}\text{min}^{-1}\text{pM}^{-1} \), was computed with the \( V_G \) values used in the parameter estimation.

The gastric delivery rate \( k \) in controls is in the range of values found by Schirra et al. (35) and is larger in T2D patients (see Tables 1 and 2). The coefficient of intestinal absorption, \( \Gamma \), is found to be markedly larger in T2D patients, according to the observation that in experimental diabetes as well as in fat-induced insulin-resistant states the apical GLUT2 fraction remains permanently high (25, 38). The values of the minimal model parameters are also in the range reported in the literature (7, 9, 10, 34), with the insulin sensitivity largely decreased in T2D patients. The glucose that entered the circulation from 0 to 180 min (AUC of \( R_3 \)) is found to be 76.1 ± 1% of the dose in controls and 78.1 ± 0.9% in T2D patients (see Refs. 19 and 20). The smaller \( GLP_b \) and \( bGLP \) values in T2D patients agree with our previous finding of a smaller GLP-1 AUC in these patients (34). The \( bGLP \) estimated derived from the intraduodenal infusion experiment, taking \( y_L = y \) and \( aGLP \) as above, are \( 2.6 \times 10^{-9} \text{L}^{-1} \text{min}^{-1} \text{pM}^{-1} \) from data in Schirra et al. (35) and \( 6.7 \times 10^{-9} \text{L}^{-1} \text{min}^{-1} \text{pM}^{-1} \) from data in Chaikinom et al. (6). These values are much smaller than the OGTT \( bGLP \) of Table 1. However, we note that the active (not the total) GLP-1 was obtained with the \( R_3 \) of the reference parameters is given in the following. As expected, GLP\(_b \) was minimally affected in all cases, with deviations <7%. Larger deviations in the other estimates were caused by changes in \( z_1 \) and \( z_2 \), as shown in Fig. 4A for \( z_2 \). The maximal deviations found in the estimates were as follows: −20% in \( k \) with \( z_1 = 10 \); 12% in \( \Gamma \) with \( c_1/c_2 = 0.4 \); −15.8% in \( S_G \) with \( z_2 = 420 \); 9.3% in \( S_I \) with \( z_2 = 280 \); 7.1% in \( p \) with \( z_2 = 280 \); −18.6% in \( bGLP \) with \( z_1 = 10 \). Deviations <15% were caused by changes in \( \beta \), \( u \), \( \sigma_1 \), \( \sigma_2 \), and \( c_1/c_2 \). Within the limits of this analysis, with the \( a \) priori parameters changed one at a time, all the deviations were <10% for changes of ±10%.

A different aspect of sensitivity concerns the hypothesis that the rate of GLP-1 secretion in Eq. 7 is proportional to the rate of glucose uptake by \( L \) cells as opposed to the luminal glucose concentration. To compare the two assumptions, the model was fitted to the average data with \( y_L(z) = y(z) \) or \( y_L(z) = 1 \). The time courses of GLP-1 concentration obtained in the two cases are reported in Fig. 4B and show that the model with \( y_L(z) = 1 \) fails to fit adequately the data. The biphasic GLP-1 concentration time course seems to require, at least for the present data, a biphasic profile of \( y_L(z) \).

Model validation was assessed in two ways. First, we verified whether the model, with the parameters set at the values estimated without considering the GIP data (mean values of Tables 1 and 2), was able to predict the experimental time course of GIP concentration in controls and T2D patients. GIP profile was computed by Eq. 6, with \( aGIP = (\ln 2)/7 \text{min}^{-1} \) (12), \( bGIP \) set to a value such that model-predicted GIP AUC equals that of the data, and \( y_G(z) = y(z) \) for \( z ≤ 250 \text{ cm} \) (the region where most of K cells are found) and then decreased to zero. The predicted GIP concentration time course for controls and T2D patients appear to adequately represent the data, as shown in Fig. 4C.

As a further validation test, the clamp-derived measurements \([M/(G_b \times \Delta t)]\) and the oral model estimates of the insulin sensitivity were compared. Figure 4D shows the correlation plots in log-log scale for controls and T2D patients (sensitivity values reported in Tables 1 and 2). The Spearman’s correlation coefficients are 0.929, \( P < 0.001 \), for controls and 0.886, \( P < 0.02 \), for T2D patients. The curves in Fig. 4D represent in log-log scale the linear regression equations: \( y = 0.39x + 0.61 \) (controls) and \( y = 0.55x - 0.15 \) (T2D patients).

**DISCUSSION**

The present mathematical model provides valuable information regarding gastric emptying, intestinal transit time, \( R_3 \) of the ingested glucose, and the insulin sensitivity after a routine OGTT. The insulin sensitivity index, \( S_i \), is well correlated with that obtained by the euglycemic hyperinsulinemic clamp (\( r = 0.929 \) in controls and 0.886 in T2D patients).
The major finding of the present study is that the GLP-1 kinetics is useful to estimate the glucose Ra. In fact, our model was effective in fitting the experimental OGTT glucose and GLP-1 data in both healthy subjects and T2D patients. Although the mathematical model proposed here for the OGTT analysis requires the measurement of an additional hormone concentration (GLP-1), it appears to be a reasonable choice since it avoids complex and expensive techniques, such as isotopes for the glucose Ra measurement and the direct assessment of gastric emptying and intestinal transit, and since it gives a number of correlated information, thus largely compensating for the extra expense.

The complex pattern of the Ra of exogenous glucose in the plasma has been interpreted by Dalla Man et al. (11) by assuming that the parameter that regulates the gastric emptying into the duodenum, denoted as $k_{empt}$, is a nonlinear function of the glucose content in the stomach. In particular, these authors found a large $k_{empt}$ (0.045 min$^{-1}$) in the initial and final phases of gastric emptying and a value more than three times smaller in the midphase (0.013 min$^{-1}$) (11). Although the assumption of this U-shaped coefficient might account for the GLP-1-induced delay in gastric emptying, a different explanation is that the gastric emptying is suitably represented by the power exponential function according to Schirra et al. (35) and that the rate coefficient of intestinal absorption $\gamma(z)$ is nonuniform in the different regions of the small intestine with two components, one proximal and the other one distal. According to this assumption, the Ra profile can display different forms, as shown in Fig. 2D, thus being able to reproduce the shapes observed in the experimental Ra data.

The one-dimensional convective transport equation with constant velocity and no dispersion (Eq. 1) used in this model represents a rather crude approximation for the movement of the intestinal contents driven by coordinated segmenting plus peristaltic waves of the gut wall. However, it may be adequate to describe the transport along a highly packed and circumvolved conduit, where the glucose load moves aborally while being subdivided into a sequence of spatially discrete boluses. For the sake of simplicity, the intestinal absorption of glucose has been represented by a linear equation with time-independent coefficients, although there is evidence that 1) the transporters are located in the luminal and the basolateral membrane of enterocytes and display intracellular trafficking (25, 38), 2) the transporters have different affinities and operate with different mechanisms (active uptake or facilitated diffusion), 3) other substrates (sodium) may be involved, 4) the maximal absorptive capacity of duodenum may be exceeded when glucose concentration is very high in the initial phase of gastric delivery, and 5) the velocity, $u$, of bolus transport may change during the intestinal transit. In particular, the GLUT2 recruitment to the apical membrane of enterocytes, as regulated by luminal glucose and plasma insulin concentrations (25, 38),...
involves a nonlinear absorption and a feedback loop that should be modelled by representing the rate of intestinal absorption, $\gamma$, as a function of $q(z,t)$ and of the insulin concentration in plasma. Another feedback loop derives from the modulation of gastric motility by the incretins. Thus other mechanisms, besides the transporter distribution hypothesized in the present study, might intervene in generating a complex absorption pattern. Both the insulin-induced decrease of the apical GLUT2 fraction (25, 38) as well as a regulated rate of absorption pattern. Both the insulin-induced decrease of the

Finally, we would like to stress that the major advantage of accurately modeling an oral glucose load is that of utilizing a more physiological way of glucose administration, compared with intravenous procedures like clamp or frequently sampled intravenous glucose tolerance test, and that of physiologically stimulating the secretion of incretins, which in turn act by increasing insulin delivery from pancreatic $\beta$-cells. Furthermore, an OGTT is better tolerated by subjects, it is not time consuming like the frequently sampled intravenous glucose tolerance test, and that of not requiring trained physicians or nurses as in the clamps.

APPENDIX A: TRANSPORT EQUATION

A mass conservation equation for the glucose amount moving at a constant velocity, $u$, in the lumen of an intestinal segment of length $\Delta z$ that is from $z$ to $z + \Delta z$ may be written as follows. Using standard approximations, the luminal glucose mass at time $t + \Delta t$ is equal to the mass present at time $t$ plus the mass inflow and minus the mass outflow in the time interval from $t$ to $t + \Delta t$. So we write

$$q(z, t + \Delta t) \Delta z = q(z, t) \Delta z + uq(z, t) \Delta t - uq(z + \Delta z, t) \Delta t - \gamma q(z, t) \Delta z \Delta t,$$  (A1)

where the second term in the right-hand side of Eq. A1 is the mass flow (with a common velocity of particles equal to $u$) entering the segment at the location $z$ in the time interval $(t, t + \Delta t)$, the third term if the outflow at $z + \Delta z$ is the same time interval, and the fourth term is the outflow due to the absorption in the enterocytes. Dividing both members of Eq. A1 by $\Delta z \Delta t$ and letting the increments go to zero, Eq. 1 is obtained.

APPENDIX B: EXPONENTIAL DELIVERY AND UNIFORM ABSORPTION

A closed-form expression for $R_d$ is easily derived under two simplifying assumptions. First, the gastric retention is exponential ($\beta = 1$) without an initial time lag, so $h(t) = \exp(-kt)$, and the gastric delivery rate is $\eta(t) = D_k \exp[-kt]$. Second, the absorption rate coefficient, $\gamma$, is constant with $z$.

In this situation the density, $q$, for $z \in [0, L]$, is given by

$$q(z, t) = \begin{cases} \frac{D_k}{u} \exp[ -k(t - z/u) ] \exp[ -\gamma z/u ], & 0 \leq t - z/u \\ 0, & \text{elsewhere}. \end{cases}$$  (B1)

The integration in Eq. 5 is performed from 0 to $\min[\omega, L]$, obtaining for $R_d(t)$ the equation

$$R_d(t) = D_k \frac{k \gamma}{k - \gamma} \exp(-kt) \left[ \exp( (k - \gamma) \min(\omega, L)/u ) - 1 \right].$$

Thus we get, for $k \neq \gamma$
tion at \( t = 0 \), \( S_q, S_t, V_{Gl} \), and \( p \); power exponential function: \( \beta, k; \) glucose transporters: \( z_1, z_2, \sigma_1, \sigma_2, \chi_1, \) and \( \chi_2; \) Eqs. 3 and 5: \( u, L, \) and \( \Gamma; \) Eq. 7: \( GLP, \alpha_{GLP}, \) and \( beta_{GLP}. \) Moreover, we have assumed that \( \gamma_1 = \gamma \), so there are no additional parameters.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES