Mathematical model of standard oral glucose tolerance test for characterization of insulin potentiation in health

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Abstract

Two new formulations, respectively denominated INT_M1 and INT_M2, of an integrated mathematical model to describe the glycemic and insulinemic responses to a 75 g oral glucose tolerance test (OGTT) are proposed and compared. The INT_M1 assumes a single compartment for the intestine and the derivative of a power exponential function for monophasic representation of gastric emptying rate profile. In the INT_M2, a nonlinear three-compartment system model is adopted to produce a more realistic, multiphase gastric emptying rate. Both models were implemented in a Matlab-based, two-step procedure for estimation of seven adjustable coefficients characterizing the gastric emptying rate and the incretin, insulin and glucose kinetics. Model behaviour was tested vs. data of mean plasma glucagon-like peptide 1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP), glucose and insulin concentrations provided by two different laboratories, where glycemic profiles observed during a 75 g OGTT were matched in healthy subjects (HC1- and HC2-group, respectively) by means of an isoglycemic intravenous glucose (I-IVG) infusion. Under the hypothesis of an additive effect of GLP-1 and GIP on insulin potentiation, our results demonstrated a substantial equivalence of the two models in matching the data. Model parameter estimates showed to be suitable markers of differences observed in the OGTT and
matched I-IVG responses from the HC1-group compared to the HC2-group. Model implementation in our two-step parameter estimation procedure enhances the possibility of a prospective application for individualization of the incretin effect in a single subject, when his/her data are plugged in.
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coefficient into a signal that enters the compartment of plasma incretin (INC). Similarly, $q_{gut}$ is multiplied by the $k_{abs}$ rate constant of intestinal absorption and the $f$ fraction of the intestinal absorption that actually appears into plasma, to predict the $R_{aG}$ rate of glucose absorption from the gut into the mesenteric circulation. This feeds the glucose compartment (G). Solid lines with arrows indicate directional flows. Dashed lines with arrows indicate control actions of incretin (INC) and glucose (G) on post-hepatic insulin secretion, as well as control actions of insulin (I) on hepatic glucose balance (HGB) and glucose delivery to the peripheral tissues.

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Chapter 1.

Introduction

1.1. The glucose-insulin regulatory system

In order to function properly, humans need to maintain an appropriate blood plasma glucose concentration level. The glucose housed within plasma provides the necessary energy required by cells within the body. The human body has a complex glucose-insulin regulatory system that targets and maintains a narrow range of plasma glucose concentration, a state of euglycemia. For example, after an overnight fast the human body typically needs to have glucose concentration level within the range of 70 – 110 mg/dl. When the glucose concentration falls well without of the normal range (into either a state of hyperglycemia or hypoglycemia) the body’s function is not only impaired but also put at significant risk. States of hyperglycemia and hypoglycemia can lead to retinopathy, neuropathy, peripheral neuropathy, blindness and even death. Impairment of the glucose-insulin regulatory system is cause of several metabolic derangements including insulin resistance and diabetes.
Food consumption is the most important source of glucose. To utilize the available glucose, the \( \beta \)-cells of the pancreas produce insulin, a hormone that triggers glucose consumption on a cellular level. Other sources of circulating glucose are derived chiefly from hepatic processes: glycogenolysis, the breakdown of glycogen, the polymerized storage form of glucose; and gluconeogenesis, the formation of glucose primarily from lactate and amino acids during the fasting state. Glycogenolysis and gluconeogenesis are partly under the control of glucagon, a hormone produced in the \( \alpha \)-cells of the pancreas. During the first 8 – 12 hours of fasting, glycogenolysis is the primary mechanism by which glucose is made available; glucagon facilitates this process and thus promotes glucose appearance in the circulation. Over longer periods of fasting, glucose, produced by gluconeogenesis, is released from the liver (Figure 1.1).
In the fasting state, glucose leaves the circulation at a constant rate. To keep pace with glucose disappearance, endogenous glucose production is necessary. For all practical purposes, the sole source of endogenous glucose production is the liver. Renal gluconeogenesis contributes substantially to the systemic glucose pool only during periods of extreme starvation. Although most
tissues have the ability to hydrolyze glycogen, only the liver and kidneys contain glucose-6-phosphatase, the enzyme necessary for the release of glucose into the circulation.

In the fed state, after reaching a post-meal peak, blood glucose slowly decreases during the next several hours, eventually returning to fasting levels. In the immediate post-feeding state, glucose removal into skeletal muscle and adipose tissue is driven mainly by insulin. At the same time, endogenous glucose production is suppressed by 1) the direct action of insulin, delivered via the portal vein, on the liver, and 2) the paracrine effect or direct communication within the pancreas between the α- and β-cells, which results in glucagon suppression.

Glucoregulatory hormones include, behind insulin and glucagon, amylin, glucagon-like peptide 1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP), epinephrine, cortisol, and growth hormone. Of these, amylin is derived from the pancreatic β-cells, while GLP-1 and GIP from the L and K cells of the intestine, respectively. The glucoregulatory hormones of the body are designed to maintain circulating glucose concentrations in a relatively narrow range.

1.1.1 Incretin hormones GIP and GLP-1

The concept that certain factors produced by the intestinal mucosa in response to nutrient ingestion are capable of stimulating the release of substances
from the endocrine pancreas and thereby reducing blood glucose levels was first introduced in the early 1900s [1.1, 1.2]. The term incretin subsequently was used to denote these glucose-lowering, intestinal derived factors [1.3]. With the development of the radioimmunoassay, this communication between the intestine and the endocrine pancreas was confirmed when it was shown that oral glucose administration is associated with a much greater increase in plasma insulin levels when compared with the same amount of glucose given intravenously [1.4, 1.5]. This phenomenon has been dubbed the incretin effect, and is estimated to account for approximately 50%–70% of the total insulin secreted after oral glucose administration. Thus, the incretin effect, that is the contribution of gastrointestinal factors released in response to nutrient ingestion, stimulates oral glucose dependent insulin secretion.

The first incretin hormone to be identified was isolated from crude extracts of porcine small intestine and initially was named gastric inhibitory polypeptide (GIP), based on its ability to inhibit gastric acid secretion in dogs [1.6]. However, subsequent studies using more purified preparations of GIP revealed that GIP could also stimulate insulin secretion in animals and humans. Because the inhibitory effect of GIP on gastric acid secretion was seen only at pharmacologic doses, whereas its incretin action occurred at physiologic levels, GIP was renamed glucose-dependent insulinotropic polypeptide, to reflect its physiologic action, yet retaining the acronym. In accordance with its role as an incretin hormone, GIP is
released from K-cells of the small intestine, primarily in response to glucose or fat ingestion, and potentiates glucose-stimulated insulin secretion. It was recognized, however, that GIP alone could not fully account for the incretin effect in vivo. This was based on the observations that immunoneutralization of endogenous GIP activity attenuates but does not abolish the incretin effect in rodents. In humans, surgical resection of the ileum is associated with diminished incretin activity, despite preservation of normal plasma GIP levels [1.7].

The discovery of a second incretin hormone, glucagonlike peptide-1 (GLP-1), followed the cloning and sequencing of mammalian proglucagon genes and complementary DNAs (cDNAs). In addition to glucagon, the proglucagon gene also encoded two peptides that were approximately 50% homologous to glucagon and thus aptly were named glucagon-like peptide-1 and glucagon-like peptide-2. Based on their homology to glucagon, both peptides were tested for insulinotropic activity, but only GLP-1 was capable of stimulating insulin secretion. GLP-1 is a tissue-specific post-translational proteolytic product of the proglucagon gene that is released from intestinal L-cells in response to nutrient ingestion and enhances glucose-stimulated insulin secretion [1.8-1.12].

To date, only GIP and GLP-1 fulfill the definition of an incretin hormone in humans [1.13]. These hormones are released during glucose or meal intake in proportion to nutrient transport across the intestinal epithelium [1.14], their effects seem to be additive, and they stimulate insulin secretion both at fasting and
postprandial plasma glucose levels [1.15]. Therefore, GIP and GLP-1 were shown to exert their insulinotropic effects through a variety of mechanisms, including by increasing the rates of insulin synthesis, granule docking, and exocytosis [1.16].

1.2. Incretin-induced insulin potentiation

In accordance with Nauck et al. [1.17], the quantity of β-cell secretory response evoked by factors other than glucose itself (insulin potentiation) can be deduced from the integrated incremental response (over basal) of insulinemia observed after an oral glucose tolerance test (OGTT), as it compares with the insulin response to an intravenous glucose infusion given in amount sufficient to match the profile of glucose concentration observed during OGTT (isoglycemic intravenous glucose, I-IVG, infusion) [1.18-1.20]. An insulin potentiation factor, IP, can be quantified as the percentage of the OGTT response by the equation:

\[
IP\% = \frac{AUC_{OGTT} - AUC_{I-IVG}}{AUC_{OGTT}} \times 100
\]  
(1.1)
where $\text{AUCI}_\text{OGTT}$ and $\text{AUCI}_\text{IVG}$ represent the area under the curve of incremental insulin concentration over the OGTT and the matched I-IVG duration, respectively, according to the trapezoidal rule.

Considering that C-peptide is co-secreted with insulin in equimolar concentration, is not extracted by any appreciable extent by the liver, and has a constant metabolic clearance rate over a wide range of physiological serum concentration, a model of C-peptide secretion and kinetics has recently been used by Campioni et al. [1.20] to assess the incretin-induced insulin potentiation and determine whether the incretin effect results from an increase in the dynamic response to glucose (i.e., the response to a change in glucose concentration), static response to glucose (the response to a given glucose concentration) or a combination of both. Application of C-peptide model to OGTT and matched I-IVG data, demonstrated that incretin effect increases insulin secretion by enhancing both the dynamic and static response to glucose. Since increases in the dynamic response to glucose are believed to be due to an increase in the rate of docking and exocytosis of insulin containing granules and the static response to glucose are believed to be caused by a shift in the sensitivity of the $\beta$-cell to glucose, these confirm that incretins modulate more than one step in the $\beta$-cell insulin secretory cascade.

Quantification of the ability of incretin effect to enhance the dynamic and static response is important to evaluate impairment of this function in subject with
underlying defects in insulin secretion. GLP-1 secretion is reduced in patients with type 2 diabetes both during an oral glucose load [1.21] and during a meal test compared with lean or obese nondiabetic subjects [1.22], whereas stimulation of insulin secretion by acute or extended GLP-1 infusions is relatively well preserved in diabetic patients [1.23, 1.24]. In first-degree relatives of type 2 diabetic patients, the incretin effect, calculated as the difference between oral glucose loading and isoglycemic intravenous glucose administration, on insulin secretion has been reported to be normal, and the plasma GLP-1 and GIP concentrations after the oral test have been found to be normal or even increased [1.18, 1.24-1.26]. In subjects with impaired glucose tolerance (IGT), β-cell function is impaired, whereas the incretin effect is only partially affected [1.19].

1.3. Integrated models of glucose-insulin system, incorporating incretin effect

Mathematical modeling in the assessment of insulin-glucose interactions has a longstanding tradition, and reported models show a wide degree of complexity depending on their purpose. Besides models of minimal complexity, some of which are referred to as “minimal models”, after Bergman et al. [1.27],
increasing relevance is being assumed by integrated simulation models of the glucose-insulin control system, during an oral glucose tolerance test (OGTT) or meal glucose tolerance test (MGTT), to improve knowledge of diabetes pathophysiology and assess the efficacy of hypoglycemic agents in clinical drug development [1.28-1.32]. In this context, a key issue concerns the modeling of glucose transit through the gastro-intestinal tract for description of glucose absorption [1.33-1.35] and the related “incretin effect” [1.1-1.5, 1.14, 1.15, 1.17-1.20, 1.34, 1.36-1.39]. To our knowledge, this aspect has been integrated in different ways in the models proposed by Silber et al. [1.32] and Brubaker et al. [1.30].

The integrated glucose-insulin model of oral glucose tolerance test, in healthy volunteers, proposed by Silber et al. [1.32] (Figure 1.2), is an extension of a previously developed model applicable to intravenous glucose tolerance test data [1.40]. Applicability to OGTT data is made possible by incorporating mathematical descriptions of glucose absorption and of incretin effect on insulin secretion. Especially, the incretin effect is incorporated as a direct effect on insulin secretion and a linear function is used to link this effect to the amount of glucose in the absorption compartment. Unfortunately, in an attempt to implement this model we found a drawback in that the empiric flexible input function used to describe glucose absorption was hardly reproducible.
A more promising simulation model of the oral glucose tolerance test, primarily intended to illustrate the importance of incretin within the normal ranges observed clinically in humans, was that proposed by Brubaker et al. [1.30] (Figure 1.3).
As reported in [1.30], glucose entry into the body during an OGTT involves two main compartments: the gastrointestinal (GI) tract and the mesenteric circulation. Because it was not the explicit intent of their program to model the GI tract, a simple linear function was used to approximate the profiles (Duod$_G$) of 50 and 100 g oral glucose loads emptying into the gut (Equations 1.2 and 1.3, respectively), as previously reported by Schirra et al. [1.34]:

$$
\text{Duod}_G (50 \text{ g}) = \begin{cases} 
0 & t < 5 \\
3.854 - 0.0261 t & 5 \leq t \leq t_{\text{max} \ 50\text{g}} \\
0 & t > t_{\text{max} \ 50\text{g}}
\end{cases}
$$

(1.2)
\[ Duod_G(100 \text{ g}) = \begin{cases} 
0 & t < 5 \\
6.349 - 0.0353 t & 5 \leq t \leq t_{\text{max} \text{ 100g}} \\
0 & t > t_{\text{max} \text{ 100g}} 
\end{cases} \quad (1.3) \]

where \( t_{\text{max} \text{ 50g}} = 147.7 \text{ min} \) and \( t_{\text{max} \text{ 100g}} = 179.9 \text{ min} \).

Following entry of glucose into the gut, the rate of absorption of glucose into the mesenteric circulation (\( Ra_{\text{GutG}} \)) was described by an empirical time-weighted exponential function adapted to data previously reported by Ferrannini et al. [1.41, 1.42] for 50 g (Equation 1.4) and 100 g (Equation 1.5):

\[ Ra_{\text{GutG}}(50 \text{ g}) = \begin{cases} 
0 & t < 5 \text{ min} \\
0.255 (t-5)^{0.106} e^{-0.035 (t-5)} & t \geq 5 \text{ min} 
\end{cases} \quad (1.4) \]

\[ Ra_{\text{GutG}}(100 \text{ g}) = \begin{cases} 
0 & t < 5 \text{ min} \\
0.36 (t-5)^{0.05} e^{0.029 (t-5)} & t \geq 5 \text{ min} 
\end{cases} \quad (1.5) \]

Unfortunately, this simple empiric description of the rate of gastric emptying of ingested glucose into the gut, \( Duod_G \), constrains the model to the reproduction of glycemias and insulinemia responses to 50 and 100 g oral glucose.
loads, and does not allow an effective model testing versus data measured during clinical OGTT protocols, which generally consist of the administration of a standard 75 g glucose dose. Moreover, as the contribution of intestinal hormones to oral glucose-stimulated insulin secretion is relevant, and being the major stimulus to the release of these hormones clearly related to the presence of glucose in the upper GI tract, oversimplification of the mathematical description of DuodG and the related rate of glucose appearance RaGutG adopted by Brubaker et al. [1.30] may limit the ability of the simulation model to capture verisimilar shapes of insulinemia and glycemia profiles during OGTT. A further critical point is that, in its original formulation [1.30], the model is characterized by fifteen parameters, ten of which are given numerical values known a priori from previously reported measurements, thus leaving five “adjustable” parameters, which were presumably manually adjusted. The lack of a validation against standard OGTT data limits the reliability of the values assumed for these “adjustable” parameters, thus contributing to affect the predictive capabilities of the model. All variables and parameter values reported by Brubaker et al. [1.30] are summarized in Tables 1.1 and 1.2, respectively.
Table 1.1. Description, basal values and units for variables used in [1.30].

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
<th>Basal value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>Plasma glucose concentration</td>
<td>4</td>
<td>mmol·L⁻¹</td>
</tr>
<tr>
<td>Inc</td>
<td>Plasma incretin concentration (GIP + GLP1)</td>
<td>200</td>
<td>ng·L⁻¹</td>
</tr>
<tr>
<td>I</td>
<td>Plasma insulin concentration</td>
<td>10</td>
<td>mU·L⁻¹</td>
</tr>
<tr>
<td>Hepbal₂G</td>
<td>Net hepatic G balance</td>
<td>0.8549</td>
<td>mmol·min⁻¹</td>
</tr>
<tr>
<td>Duod₂G</td>
<td>Rate of appearance of ingested glucose in the duodenum</td>
<td>0</td>
<td>mmol·min⁻¹</td>
</tr>
<tr>
<td>RaGutG</td>
<td>Rate of appearance of Duod₂G in the blood</td>
<td>0</td>
<td>mmol·min⁻¹</td>
</tr>
<tr>
<td>t</td>
<td>Time</td>
<td>0</td>
<td>min</td>
</tr>
</tbody>
</table>

Table 1.2. Description, values and units for parameters used in [1.30].

<table>
<thead>
<tr>
<th>Constant</th>
<th>Description</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>k₁</td>
<td>Non-insulin mediated glucose uptake (NIMGU)</td>
<td>0.00671</td>
<td>L⁰·³·mmol⁻⁰·³·min⁻¹</td>
</tr>
<tr>
<td>k₂</td>
<td>Insulin-mediated glucose uptake (IMGU)</td>
<td>0.00204</td>
<td>mmol·min⁻¹·mU⁻¹</td>
</tr>
<tr>
<td>k₃</td>
<td>Slope of renal glucose clearance</td>
<td>0.0718</td>
<td>L·min⁻¹</td>
</tr>
<tr>
<td>k₄</td>
<td>Intercept of renal glucose clearance</td>
<td>0.717</td>
<td>mmol·min⁻¹</td>
</tr>
<tr>
<td>k₅</td>
<td>Rate of appearance of Inc due to Duod₂G</td>
<td>27.64</td>
<td>ng·L⁻¹·mmol⁻¹</td>
</tr>
<tr>
<td>k₆</td>
<td>Measure of degradation/clearance of Inc</td>
<td>0.1</td>
<td>min⁻¹</td>
</tr>
<tr>
<td>k₇</td>
<td>Rate of appearance of I due to G</td>
<td>0.125</td>
<td>mU·min⁻¹·mmol⁻¹·L⁻⁰·³</td>
</tr>
<tr>
<td>k₈</td>
<td>Rate of appearance of I due to Inc</td>
<td>0.005</td>
<td>mU·min⁻¹·ng⁻¹</td>
</tr>
<tr>
<td>k₉</td>
<td>Measure of degradation/clearance of I</td>
<td>0.1</td>
<td>min⁻¹</td>
</tr>
<tr>
<td>M</td>
<td>Effects of counter-regulatory factors on liver</td>
<td>0.02 (M=0.03 if G &lt; 3)</td>
<td>L²·mU⁻¹·min⁻¹</td>
</tr>
<tr>
<td>RaInc</td>
<td>Rate of appearance of Inc</td>
<td>280</td>
<td>ng·min⁻¹</td>
</tr>
<tr>
<td>V</td>
<td>Volume of distribution</td>
<td>14</td>
<td>L</td>
</tr>
<tr>
<td>α</td>
<td>Hepatic regulatory term for hypoinsulinemia</td>
<td>1.0</td>
<td>mmol²·mU·L⁻²·min⁻¹</td>
</tr>
<tr>
<td>β</td>
<td>Effects of additional regulators of I on insulin</td>
<td>-0.758</td>
<td>mU·L⁻¹·min⁻¹</td>
</tr>
<tr>
<td>γ</td>
<td>Shaping factor for derivative control of insulin on glucose</td>
<td>0.06 if RaGutG &gt; 0</td>
<td>mmol·mU⁻¹</td>
</tr>
</tbody>
</table>
In an attempt to test the quality of Brubaker’s model in simulating the time course of insulinemia and glycemia responses to OGTT, we implemented the model in a Matlab environment and ran it with the Duod$_G$ and Ra$_{GutG}$ functions described by Equations 1.2 to 1.5 and the parameterization reported in Table 1.1 and 1.2. As it is shown in Figure 1.4, simulated glycemia and insulinemia profiles in response to 50 g and 100 g glucose loads were superimposed to mean glycemia and insulinemia data reported by Campioni et al. [1.20] from 75 g OGTT responses of 10 healthy subjects. Limitations in model behavior are evident in that 1) in Figure 1.4 A, both simulated glycemia profiles in response to 50 g (dotted line) and 100g (dashed line) glucose load are characterized by an early peak followed by a rapid decrease, that are significantly underestimated with respect to the profile of mean glycemia data (closed squares) from standard OGTT, and 2) in Figure 1.4 B, the simulated insulinemia response to 50 g glucose load (dotted line) approximates the mean insulinemia data (closed circle) from standard 75 g OGTT, while the peak of simulated insulinemia response to 100 g glucose load (dashed line) is almost twice as large as the peak observed in the insulinemia data.
Figure 1.4. Model simulations of glycemia (panel A) and insulinemia (panel B) profiles in response to 50 g (dotted lines) and 100 g (dashed lines) glucose loads are superimposed to mean glycemia (closed squares in panel A) and insulinemia (closed circles in panel B) data reported by Campioni et al. [1.20] from 75 g OGTT responses of 10 healthy subjects.
1.4. Mathematical models of oral glucose absorption

Oral ingestion of glucose is used in everyday meals as well as in the most important clinical test to assess glucose tolerance in humans, through the oral glucose tolerance test (OGTT). After ingestion, glucose is absorbed in the upper gastrointestinal tract, transported to the splanchnic bed (mostly the liver) and, finally, reaches the peripheral circulation.

A model describing or mimicking the mechanisms of glucose transit through the gastrointestinal tract would be very useful to study possible abnormalities of glucose absorption in particular populations, e.g., elderly versus young and diabetic versus normal individuals, as well as validating simulation models of glucose regulation. In fact, only a few of the currently available simulation models allow an oral route of glucose administration [1.43-1.46] but in all, the glucose absorption process is described rather simplistically. Modeling glucose oral ingestion has been difficult because of lack of gold standard validation data. In fact, if such a model is tested only on plasma glucose concentrations, there is the need to append to the glucose absorption model a whole-body model of glucose kinetics and their hormonal control. Under such assumptions, model error compensations are likely to occur and yet go undetected. This is because a good fit of the plasma glucose concentration can easily mask possible model errors in the
glucose absorption, as these errors are compensated by errors in the whole-body
description of glucose kinetics.

The gold standard data to test a glucose absorption model would be
provided by detailed knowledge of the time course of appearance in plasma of
ingested glucose (Ra). This would allow avoidance of the model of whole-body
glucose kinetics. However, reliable model-independent knowledge of Ra requires
the use of multiple tracer, oral glucose protocols implementing the tracer-to-tracee
ratio clamp technique [1.47].

Only recently these data have become available [1.47, 1.48] and have been
utilized to propose and validate glucose absorption models, based on the reported
glucose appearance (Ra) data that are used as the gold standard.

Making use of this new data set Dalla Man et al. [1.35] tested two
previously published models [1.33, 1.44, 1.45], respectively denoted as Lehmann
& Deutsch model and Elashoff et al. model, and two new models denoted as Model
1 and Model 2, respectively. The Lehmann & Deutsch model [1.44] assumes a
trapezoidal gastric emptying function, a single compartment for the intestine and a
constant rate of intestinal absorption, while the Elashoff et al. model [1.33]
assumes a power exponential gastric emptying function, a single compartment for
the intestine and a constant rate of intestinal absorption. Model 1 and Model 2
[1.35] are more complex in that they consist of a chain of three compartments: the
first two represent the stomach, while the third one represents the intestine. The
difference between the latter two formulations is in that Model 1 is linear, while Model 2 is nonlinear. In the linear model formulation, both the rate of intestinal absorption ($k_{abs}$) and the gastric emptying rate ($k_{empt}$) are assumed to be constant. In the nonlinear formulation, $k_{abs}$ is constant while $k_{empt}$ is dependent on the total amount of glucose in the stomach, $q_{sto} [1.49-1.55]$ (equation 1.6):

$$k_{empt}(q_{sto}) = k_{min} + \frac{k_{max} - k_{min}}{2} \cdot \left\{ \tanh[e_1 \cdot (q_{sto} - b \cdot D)] - \tanh[e_2 \cdot (q_{sto} - c \cdot D)] + 2 \right\} \quad (1.6)$$

A better grasp of the parametric control of $q_{sto}$ on $k_{empt}$ can be obtained by its graphical representation (Figure 1.5).
Figure 1.5: Qualitative plot of gastric emptying rate ($k_{empt}$) as function of the amount of glucose in the stomach $q_{stom}$ [1.35].

Equation (1.6) yields a multiphase representation of the rate of appearance of glucose (solid line in the bottom panel of Figure 1.6), which approximates the gold standard data (solid circles) better than the monophase representations provided by the Model 1 (dashed line in the bottom panel of Figure 1.6), the Lehmann & Deutsch model (dashed line in the upper panel of Figure 1.6) and the Elashoff model (solid line in the upper panel of Figure 1.6).
Figure 1.6. Upper panel: data versus predictions during OGTT of Lehmann & Deutsch model and Elashoff et al. model; lower panel: data versus predictions during OGTT of Model 1 and Model 2 [1.35].

1.5. Aims of the thesis

As the contribution of intestinal hormones to oral glucose-stimulated insulin secretion is relevant, and being the major stimulus to the release of these hormones clearly related to the presence of glucose in the upper gastrointestinal tract, we considered that a more realistic description of glucose absorption as
deduced from the comparative analysis reported by Dalla Man et al. [1.35] was essential to overcome the limitations affecting the Brubaker et al. model [1.30] of oral glucose tolerance test, and accomplish the goal of a more reliable representation of the augmented glucose-dependent insulin secretion, generally referred to as incretin-induced insulin potentiation.

Two alternative formulations of glucose absorption were, then, considered. Namely, the Elashoff et al. model [1.33], hereafter referred to as M1, and the nonlinear three-compartment model denominated Model 2 by Dalla Man et al. [1.35] and hereafter referred to as M2. The integrated mathematical models of standard oral glucose tolerance test obtained from incorporating the M1 and the M2 representations of glucose absorption are referred to as INT_M1 and INT_M2.

With the aim of assessing optimal values for “adjustable” coefficients characterizing the gastric emptying rate and the incretin, insulin and glucose kinetics, both the INT_M1 and INT_M2 were implemented in a Matlab-based, two-step identification procedure. Models behaviour was tested vs. mean plasma concentrations of glucagon-like peptide 1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP), glucose and insulin from two different laboratories, where glycemic profiles observed during a standard 75 g OGTT were matched in healthy subjects (HC1- and HC2-group, respectively) by means of an isoglycemic intravenous glucose (I-IVG) infusion. Especially, a comparative analysis of INT_M1 and INT_M2 behavior was performed in terms of model
ability to reproduce incretin-induced insulin potentiation, observed after OGTT, compared to that observed during the matched I-IVG infusion.
Chapter 2.

Integrated mathematical model of standard OGTT incorporating incretin effect: role of glucose absorption modeling

2.1 Model formulation

The model previously proposed by Brubaker et al. [1.30] to simulate the glycemic and insulinemic responses to a 50 g and a 100 g oral glucose load, with explicit incorporation of incretin action, was used as a basis to build-up an integrated model upon an OGTT protocol consisting of oral administration of a standard 75 g oral glucose load (i.e. 1 g of glucose per kg body weight, BW, administered at time t=0 min, and standardized to a 75 kg individual). To this aim the description of the oral glucose absorption was modified by incorporating either a one-compartment model [1.33], denoted as M1, or a three-compartment model [1.35], denoted as M2. The two versions of our integrated model, the flow diagram of which is depicted in Figure 2.1, were denoted as INT_M1 when incorporating
the M1, and INT_M2 when incorporating the M2, respectively. The overall mathematical description is reported in the following sections.

Description, basal values and units for both the INT_M1 and the INT_M2 variables are given in Table 2.1.

Table 2.1. Description, basal values and units for model variables.

<table>
<thead>
<tr>
<th>Constant</th>
<th>Description</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>$q_{sto1b}$</td>
<td>Amount of glucose in the compartment 1 of stomach.</td>
<td>0</td>
<td>mmol</td>
</tr>
<tr>
<td>$q_{sto2b}$</td>
<td>Amount of glucose in the compartment 2 of stomach.</td>
<td>0</td>
<td>mmol</td>
</tr>
<tr>
<td>$q_{gutb}$</td>
<td>Glucose mass in the intestine.</td>
<td>0</td>
<td>mmol</td>
</tr>
<tr>
<td>$G_b$</td>
<td>Plasma glucose concentration.</td>
<td></td>
<td>mmol·L$^{-1}$</td>
</tr>
<tr>
<td>$I_b$</td>
<td>Plasma insulin concentration.</td>
<td></td>
<td>mU·L$^{-1}$</td>
</tr>
<tr>
<td>$INC_b$</td>
<td>Plasma incretin concentration.</td>
<td></td>
<td>ng·L$^{-1}$</td>
</tr>
<tr>
<td>$HGB_b$</td>
<td>Hepatic glucose balance.</td>
<td>0.77</td>
<td>mmol·min$^{-1}$</td>
</tr>
<tr>
<td>$G_{emptb}$</td>
<td>Rate of emptying of glucose load into the gut.</td>
<td>0</td>
<td>mmol·min$^{-1}$</td>
</tr>
<tr>
<td>$R_{acb}$</td>
<td>Rate of appearance of glucose in the peripheral circulation.</td>
<td>0</td>
<td>mmol·min$^{-1}$</td>
</tr>
</tbody>
</table>
Figure 2.1. Flow diagram of our integrated model incorporating either one of two alternative formulations of glucose absorption, denoted as M1 and M2. The M1 representation assumes the derivative of a power exponential function for the gastric
emptying rate (Gempt) of the oral glucose dose (D), and a single compartment for the gut (qgut). In the M2 configuration, the oral glucose dose enters a two compartment model (qsto1 and qsto2) of the stomach, while a gastric emptying function (kempt), dependent on the total amount of glucose in the stomach (qsto, equal to the sum of qsto1 and qsto2), plays a key role in determining the time course of both the rate of emptying of oral glucose load into the gut (Gempt) and the glucose quantity in the gut compartment (qgut). In both our integrated model formulations (denominated INT_M1 and INT_M2, respectively), the Gempt is transmuted by the k5 coefficient into a signal that enters the compartment of plasma incretin (INC). Similarly, qgut is multiplied by the kabs rate constant of intestinal absorption and the f fraction of the intestinal absorption that actually appears into plasma, to predict the RaG rate of glucose absorption from the gut into the mesenteric circulation. This feeds the glucose compartment (G). Solid lines with arrows indicate directional flows. Dashed lines with arrows indicate control actions of incretin (INC) and glucose (G) on post-hepatic insulin secretion, as well as control actions of insulin (I) on hepatic glucose balance (HGB) and glucose delivery to the peripheral tissues.

2.1.1 M1 model of oral glucose absorption

This model describes glucose absorption by the gut as shown in the M1 panel of Figure 2.1. Under the assumption that, after ingestion of a glucose dose, D (75 g, equivalent to 417 mmol), the fraction of glucose in the duodenum follows a power exponential function increase, the rate of emptying of the oral glucose load
into the gut, \( G_{\text{empt}} \) (mmol·min\(^{-1}\)), is described by the following equation [1.33, 1.35]:

\[
G_{\text{empt}}(t) = D \cdot \beta \cdot k_e \cdot t^{\beta-1} \cdot e^{-(t \cdot \beta)}
\]  

(2.1)

where \( k_e \) (min\(^{-1}\)) is a fractional transfer coefficient and \( \beta \) a dimensionless shape factor. Denoting the amount of glucose in the gut as \( q_{\text{gut}} \) (mmol), the rate constant of intestinal absorption as \( k_{\text{abs}} \) (min\(^{-1}\)), the fraction of the intestinal absorption which actually appears in plasma as \( f \), and the rate of appearance of ingested glucose into plasma as \( R_{A_G} \) (mmol·min\(^{-1}\)), the following equations hold [1.33, 1.35]:

\[
\dot{q}_{\text{gut}}(t) = -k_{\text{abs}} \cdot q_{\text{gut}}(t) + G_{\text{empt}}(t)
\]  

(2.2)

\[
R_{A_G}(t) = f \cdot k_{\text{abs}} \cdot q_{\text{gut}}
\]  

(2.3)

### 2.1.2 M2 model of oral glucose absorption

The representation of glucose absorption displayed in the M2 panel of Figure 2.1 assumes two compartments (\( q_{\text{sto1}} \) and \( q_{\text{sto2}} \), respectively) for the stomach,
and a single compartment, $q_{gut}$, for the intestine. Mathematical description is as follows [1.35]:

\[
\dot{q}_{100}(t) = -k_{21} \cdot q_{100}(t) + D \cdot \delta(t) \tag{2.4}
\]

\[
q_{202}(t) = -k_{empt}(q_{100}) \cdot q_{202}(t) + k_{21} \cdot q_{100}(t) \tag{2.5}
\]

\[
q_{gut}(t) = -k_{abs} \cdot q_{gut}(t) + k_{empt}(q_{100}) \cdot q_{202}(t) \tag{2.6}
\]

\[
q_{100}(t) = q_{100}(t) + q_{202}(t) \tag{2.7}
\]

\[
G_{empt}(t) = k_{empt}(q_{100}) \cdot q_{202}(t) \tag{2.8}
\]

\[
Ra_{gut}(t) = f \cdot k_{abs} \cdot q_{gut}(t) \tag{2.9}
\]

with the initial conditions being the basal values $q_{100b}$, $q_{202b}$ and $q_{gutb}$ reported in Table 2.1.

In these equations $\delta(t)$ is the impulse function, $D$ (mmol) is the amount of ingested glucose, $k_{21}$ (min$^{-1}$) is the rate of grinding, $G_{empt}$ (mmol·min$^{-1}$) is the rate
of emptying of the oral glucose load into the gut, $R_{aG}$ (mmol·min$^{-1}$) is the rate of glucose absorption from the gut into the mesenteric circulation; $k_{abs}$ (min$^{-1}$) is the rate constant of intestinal absorption, $f$ is the fraction of the intestinal absorption which actually appears in the plasma, and $k_{empt}$ (min$^{-3}$) is a gastric emptying function, which depends on the total amount of glucose in the stomach, $q_{sto}$ (mmol), as follows [1.35]:

$$k_{empt}(q_{sto}) = k_{min} + \frac{k_{max} - k_{min}}{2} \cdot \{\tanh[e_1 \cdot (q_{sto} - b \cdot D)] - \tanh[e_2 \cdot (q_{sto} - c \cdot D)] + 2\} \quad (2.10)$$

As reported by Dalla Man et al. [1.35], this model is suitable to describe glucose absorption during an OGTT under the assumption of $k_{empt} = k_{max}$ at $q_{sto} = D$ (full stomach) and at $q_{sto} = 0$ (empty stomach). This assumption yields the following expressions for the $e_1$ and $e_2$ parameters:

$$e_1 = \frac{5}{2 \cdot D \cdot (1 - b)} \quad (2.11)$$

$$e_2 = \frac{5}{2 \cdot D \cdot c} \quad (2.12)$$
2.1.3 Hepatic glucose balance

Hepatic glucose balance (HGB; mmol·min⁻¹) represents the net flux of glucose G (mmol·L⁻¹) across the hepatic bed, thereby reflecting the sum of glucose production and glucose uptake from the mesenteric circulation (e.g. following the oral glucose load). In accordance with Brubaker et. al. [1.30] the following equation is assumed to describe the HGB(t) dynamics in healthy conditions:

\[
HGB(t) = HGB_b + M \cdot [G_b - G(t)] \cdot I(t)
\]

(2.13)

In this equation, HGB_b is basal hepatic glucose balance, G_b is basal glucose concentration, while G(t) and I(t) represent the time course of glucose and insulin concentrations. M is a modulating factor in the bilinear control term.

2.1.4 Incretin response

The following differential equation was assumed to describe the kinetics of the incretin [1.30]:

\[
\frac{dINC(t)}{dt} = \frac{Ra_{INC}}{V} + k_s \cdot G_{impel}(t) - k_e \cdot INC(t)
\]

(2.14)
According to this equation, three terms determine the rate of variation with time of circulating incretin concentrations (INC), based on the combined contribution of glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) [1.10-1.12, 1.15, 1.19, 1.34, 1.38]. The first term is the basal rate of appearance of the incretin (Ra_{INCb}; ng·min$^{-1}$), normalized to distribution volume (V); the second term is proportional, through the $k_3$ constant (ng L$^{-1}$·mmol$^{-1}$), to the profile of the rate of gastric emptying into the gut (G_{empt}; mmol·min$^{-1}$); and, eventually the third term accounts for a negative contribution, proportional through the $k_6$ (min$^{-1}$) to the time course of the incretin (INC; ng·L$^{-1}$) due to the removal of bioactive GIP and GLP-1 from the circulation [2.1]. The quantity

$$Ra_{INCb} = k_6 \cdot V \cdot INC_b$$  \hspace{1cm} (2.15)

is obtained from Equation 2.14 by setting the derivative equal to zero, which occurs when $G_{empt}$ equals zero.

2.1.5 Insulin response

The following differential equation was assumed to describe insulin kinetics [1.30]:

33
Equation 2.16 is based on the assumption that four terms determine the rate of variation with time of circulating insulin. The first nonlinear term, assumed equal to $k_7 \cdot G(t)^{1.3}$, accelerates the effect of plasma glucose on insulin release, with respect to a linear term; the second and the third terms are proportional, through the $k_8$ (mU·min$^{-1}$·ng$^{-1}$) and $k_9$ (min$^{-1}$) constants, respectively, to the circulating concentrations of the incretin and insulin; and eventually, the constant term, $\lambda$ (mU·L$^{-1}$·min$^{-1}$), which is expressed, in steady state, in terms of the known basal values of $G$ and $I$, after setting the derivative term, $dI/dt$, equal to zero and solving for $\lambda$:

$$\lambda = k_9 \cdot I_b - k_8 \cdot INC_b - k_7 \cdot G_b^{1.3}$$  \hspace{1cm} (2.17)

2.1.6 Plasma glucose kinetics

The following differential equation was assumed to describe the glucose kinetics [1.30]:
According to this equation, the rate of change of glucose concentration during an OGTT depends on glucose absorption from the gut into the mesenteric circulation \((Ra_G(t)/V)\); net absorption/production of glucose by the liver \((HGB(t)/V)\); insulin mediated glucose uptake, \(k_2 \cdot I(t)\); and a small corrective term, \(\gamma \cdot dI(t)/dt\), which has the effect of sharpening the peak in glucose level following entry of glucose into the circulation. A linear, \(k_1 \cdot G(t)\), non-insulin mediated glucose uptake was assumed here, rather than the \(k_1 \cdot G(t)^{1.3}\) power term originally assumed by Brubaker et al. [1.30]. Under these assumptions, denoting as \(p\) the ratio of non-insulin mediated to insulin mediated glucose uptake, and assuming a value of 2 for basal conditions [2.2, 2.3], the following relation holds:

\[
p = \frac{k_1 \cdot G_b}{k_2 \cdot I_b} = 2
\]

(2.19)

From Equation 2.18 written for steady state conditions \((dG/dt = 0\) and \(dI/dt = 0\)) and Equation 2.19, the values of the constants \(k_1\) (min\(^{-1}\)) and \(k_2\) (mmol·min\(^{-1}\)·mU\(^{-1}\)) are given by the following equations [1.30]:

\[
\frac{dG(t)}{dt} = \frac{Ra_G(t)}{V} + \frac{HGB(t)}{V} - k_1 \cdot G(t) - k_2 \cdot I(t) + \gamma \cdot \frac{dI(t)}{dt}
\]

(2.18)
\[
k_i = \frac{p}{p+1} \frac{HGB_b}{G_b \cdot V}
\] (2.20)

\[
k_2 = \frac{1}{p+1} \frac{HGB_b}{I_b \cdot V}
\] (2.21)

As reported by Brubaker et al. [1.30], the Equation 2.18 works well under normoglycemic conditions. To account for urinary glucose loss during hyperglycemia (\(G > 10 \text{ mmol} \cdot \text{L}^{-1}\)), an additional term is added as shown in the following equation:

\[
\frac{dG(t)}{dt} = \frac{Ra_G(t)}{V} + \frac{HGB(t)}{V} - k_1 \cdot G(t) - k_2 \cdot I(t) + \gamma \cdot \frac{dI(t)}{dt} \cdot \frac{k_3 \cdot G(t) - k_4}{V}
\] (2.22)

2.1.7 Model parameterization

In the absence of measurements of basal hepatic glucose balance, \(HGB_b\), the value of \(0.77 \text{ mmol} \cdot \text{min}^{-1}\) was deduced from the literature by multiplying the BW of 75 kg times \(10.25 \times 10^{-3} \text{ mmol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}\) determined as the mean of two hepatic glucose balance values per unit BW reported by Bell et al. [2.4] (and summarized at points 3 and 4 of Table A.1 in [2.5]) for humans with mean glycemia of \(5.28 \text{ mmol} \cdot \text{L}^{-1}\) and mean insulinemia of \(10 \text{ mU} \cdot \text{L}^{-1}\), which are
consistent with the mean fasting values of glycemia and insulinemia characterizing our HC1-group (5.4 mmol·L⁻¹ and 10.2 mU·L⁻¹, respectively) and HC2-group (5.5 mmol·L⁻¹ and 7.6 mU·L⁻¹, respectively).

Besides the basal values of variables as given in Table 2.1, and considered that HGBₜ is treated as a fixed parameter, the INT_M1 formulation consists of fifteen further independent parameters; namely, \( k_3, k_4, k_5, k_6, k_7, k_8, p, V, M, \gamma, k_{abs}, f, k_e \) and \( \beta \). Instead, the INT_M2 is characterized by seventeen parameters, because in this model the \( k_e \) and \( \beta \) characteristic parameters of the monophase waveshape for the rate of gastric emptying (\( G_{empt} \); Equation 2.1) are replaced by the \( c, b, k_{min} \) and \( k_{max} \) coefficients of the emptying function \( k_{empt}(q_{sto}) \) of Equation 2.10. Quantification of all these parameters was accomplished as follows. The \( k_3, k_4, k_6, k_9, p \) and \( V \) were assumed as fixed and were given numerical values (Table 2.2) known \textit{a priori} from observations reported in the literature, as discussed by Brubaker et al. [1.30]. The \( k_{abs}, f, b \) and \( c \) parameters pertaining to glucose absorption were also assumed as fixed (Table 2.2). In accordance with the validation studies on glucose-absorption models reported by Dalla Man et al. [1.35, 2.6], the value of 0.22 min⁻¹ was assumed for the rate constant, \( k_{abs} \), of intestinal glucose absorption, while the value of 0.90 was assumed for the fraction, \( f \), of the ingested glucose dose, \( D \), that is actually absorbed. Eventually, the \( b \) and \( c \) dimensionless coefficients were given the values of 0.85 and 0.25, respectively, thus fixing the percentage of the glucose dose, \( D \), for which the emptying function...
$k_{empt}$ (Equation 2.10) decreases (b) and subsequently increases (c) at $(k_{\text{max}}-k_{\text{min}})/2$ [1.35].

Table 2.2. Description, values and units for fixed INT_M1 and INT_M2 model parameters.

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Description</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{abs}$</td>
<td>Rate constant of intestinal absorption.</td>
<td>0.22</td>
<td>min$^{-1}$</td>
</tr>
<tr>
<td>f</td>
<td>Fraction of the intestinal absorption which actually appears into plasma.</td>
<td>0.90</td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>Glucose dose percentage corresponding to the first flex point of the $k_{empt}(q_{mo})$ function (eq. A10).</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>Glucose dose percentage corresponding to the second flex point of the $k_{empt}(q_{mo})$ function (eq. A10).</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>Ratio of non-insulin mediated to insulin mediated glucose uptake (NIMGU/IMGU; eq. A19).</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>$k_3$</td>
<td>Slope of renal glucose clearance.</td>
<td>0.0718</td>
<td>L·min$^{-1}$</td>
</tr>
<tr>
<td>$k_4$</td>
<td>Intercept of renal glucose clearance.</td>
<td>0.717</td>
<td>mmol·min$^{-1}$</td>
</tr>
<tr>
<td>$k_6$</td>
<td>Measure of degradation/clearance of incretin.</td>
<td>0.1</td>
<td>min$^{-1}$</td>
</tr>
<tr>
<td>$k_9$</td>
<td>Measure of degradation/clearance of insulin.</td>
<td>0.1</td>
<td>min$^{-1}$</td>
</tr>
<tr>
<td>V</td>
<td>Volume of distribution.</td>
<td>15</td>
<td>L</td>
</tr>
</tbody>
</table>

Values of $k_{abs}$, f, c and b are taken from Dalla Man et al. [1.35, 2.6]. Sources of the values given to p, $k_3$, $k_4$, $k_6$, $k_9$ and V are found in Brubaker et al.[1.30].
The remaining independent model parameters, denoted as $k_5$, $k_7$, $k_8$, $M$ and $\gamma$, which were assumed as “adjustable” in the original model of Brubaker et al. [1.30] and presumably manually adjusted, were considered as free parameters in addition to the $k_e$ and $\beta$ in the INT_M1 model formulation, and in addition to $k_{\text{min}}$ and $k_{\text{max}}$ in the INT_M2 model formulation. One novel aspect of the present work is that these free parameters were automatically estimated by a two-step procedure as described in the section 2.3.

In accordance with Dalla Man et al. [1.35], to favour numerical identifiability of the INT_M2 model, the constraint $k_{\text{21}} = k_{\text{max}}$ was imposed. After INT_M1 and INT_M2 model parameter estimation was accomplished, the remaining dependent parameters $\varepsilon_1$, $\varepsilon_2$, $\text{Ra}_{\text{INCh}}$, $\lambda$, $k_1$, and $k_2$ were computed by Equations 2.11, 2.12, 2.15, 2.17, 2.20 and 2.21, respectively.

2.2 Model testing vs. reported clinical data

Our INT_M1 and INT_M2 model outputs were tested against data of plasma glucagon-like peptide-1 (GLP-1), glucose-dependent insulino-tropic polypeptide (GIP), glucose and insulin concentration, averaged over two groups of
metabolically healthy subjects, here denominated HC1- and HC2-group, respectively reported by Muscelli et al. [1.19] and Nauck et al. [1.18].

Essentially, the subjects involved in these previous studies underwent two different glucose-challenge protocols. On the first occasion (OGTT protocol) the subject ingested 75 g of glucose, whereas on the second occasion (I-IVG protocol) an intravenous glucose infusion was given in amount sufficient to match the profile of glucose concentration observed during OGTT [1.18, 1.19]. An augmented glucose-dependent insulin secretion (insulin potentiation), observed in these circumstances after OGTT, compared to the matched I-IVG infusion, is attributed to the influence of the so called “incretin effect” mostly due to the gut-derived incretin hormones, GLP-1 and GIP, which enhance glucose-dependent insulin secretion by binding to specific receptors on the β-cell [1.8-1.12]. These hormones are released during glucose or meal intake in proportion to nutrient transport across the intestinal epithelium [1.14], their effect seems to be additive, and they stimulate insulin secretion both at fasting and postprandial plasma glucose level [1.15]. On this basis, the mean levels of GLP-1 and GIP (expressed in pmol·L⁻¹) reported in [1.19] for the HC1-group, and in [1.18] for the HC2-group, were combined as follows to define an INC(t) signal for each group. First, the reported mean levels of GLP-1 and GIP, expressed in pmol·L⁻¹, were converted into ng·L⁻¹ (conversion factors were: 1 pmol·L⁻¹ = 3.30 ng·L⁻¹ for the GLP-1, and 1 pmol·L⁻¹ = 4.98 ng·L⁻¹ for the GIP) and, then, for each group the INC(t) signal was determined as the sum
of related GLP-1 and GIP. Eventually, this INC(t), signal, was used together with the glycemia, G(t), and insulinemia, I(t), data, and the related Gb, Ib and INCb, fasting values, for model parameter estimation by the procedure described in the next section.

2.3 Parameter estimation procedure

As explained in section 2.1.7, the INT_M1 and the INT_M2 are characterized by seven free parameters, i.e., k5, k7, ks, M, γ, β and ke, the former, and k5, k7, ks, M, γ, kmin and kmax, the latter. To define an optimal value for each one of these parameters, a two-step parameter estimation procedure was set-up as follows.

The first step was to estimate the k7 parameter by running either the INT_M1 and the INT_M2 to fit mean plasma insulin concentrations from the I-IVG protocol. To this aim, Gempt (and, with it, the dynamic component of plasma incretin inflow in Equation 2.14) was set to zero, while the incretin level was maintained at the basal value, INCb, and the glucose profile matched to that measured during OGTT in the HC1- and HC2-group, respectively, was used as model input instead of the description by Equation 2.18. Under these conditions, glucose absorption is not involved, so that k7 (Equation 2.16) is the only one free
parameter that remains to be estimated from fitting the model predicted I(t) to I-IVG insulin data.

In a subsequent step, the $k_7$ estimate was filled into the INT_M1 and the INT_M2, which were run to generate incretin, glycemia and insulinemia responses to a 75 g OGTT, to be simultaneously fitted to the OGTT data (corresponding to each I-IVG), in order to estimate the six remaining unknown parameters ($k_5$, $k_8$, M, $\gamma$, $k_e$ and $\beta$) of the INT_M1, as well as the six remaining unknown parameters ($k_s$, $k_b$, M, $\gamma$, $k_{min}$, and $k_{max}$) of the INT_M2.

All model formulations were implemented in Matlab-Simulink environment. Data fit and parameter estimation were accomplished by means of a weighted least squares (WLS) procedure [2.7]. The following sum of square error (SSE) expression was used to fit the model predicted I-IVG insulin output at the i-th instant, $I_{m}(t_i)$, to the corresponding insulin measurement, $I(t_i)$:

$$\text{SSE}_{I-IVG} = \sum_{i=1}^{N} \left( \frac{I_{m}(t_i) - I(t_i)}{\Gamma_i} \right)^2$$  \hspace{1cm} (2.23)

The following SSE expression was subsequently used for simultaneous fit of model predicted OGTT incretin, $INC_{m}(t_i)$, glycemia, $G_{m}(t_i)$, and insulinemia, $I_{m}(t_i)$, outputs at the i-th instant, to the corresponding incretin, $INC(t_i)$, glycemia, $G(t_i)$, and insulinemia, $I(t_i)$, measurements:
$$\text{SSE}_{\text{OGTT}} = \sum_{i=1}^{N} \left[ \frac{(\text{INC}(t_i) - \text{INC}(t_i))}{\Gamma_{\text{INC}}} \right]^2 + \left[ \frac{G_{\text{in}}(t_i) - G(t_i)}{\Gamma_{\text{G}}} \right]^2 + \left[ \frac{I_{\text{in}}(t_i) - I(t_i)}{\Gamma_{\text{I}}} \right]^2 \right]$$ \quad (2.24)

The weights, \( \Gamma_{\text{INC}}, \Gamma_{\text{G}}, \Gamma_{\text{I}} \), were assumed equal to 5.5\%, 1.5\% and 4\% of INC\((t_i)\), G\((t_i)\) and I\((t_i)\), respectively, under the assumption of normal distribution with zero mean for the measurement errors \([1.19, 2.7]\). The goodness of fit was evaluated by calculation of the root-mean-square errors, \( \text{RMSE}_{\text{I-IVG}} = \sqrt{\frac{\text{SSE}_{\text{I-IVG}}}{N}} \) and \( \text{RMSE}_{\text{OGTT}} = \sqrt{\frac{\text{SSE}_{\text{OGTT}}}{3N}} \).

Precision of all parameter estimates was expressed as percent coefficient of variation: \( \text{CV}(p_i)\% = \frac{\text{SD}_p}{p_i} \times 100 \), where \( p_i \) is the i-th component of the model parameters vector and \( \text{SD}_p \) is the standard deviation of \( p_i \), which is calculated as the square root of the diagonal terms of the inverse of the Fisher information matrix.

2.4 Insulin potentiation

In accordance with Nauck et al. [1.17], quantification of insulin potentiation factor, IP, was deduced by the Equation 1.1 as reported in section 1.2.
2.5 Results

Mean fasting values of $G_b$, $I_b$, and $INC_b$ were $5.4 \text{ mmol} \cdot \text{L}^{-1}$, $10.2 \text{ mU} \cdot \text{L}^{-1}$, and $145 \text{ ng} \cdot \text{L}^{-1}$, respectively, for the HC1-group, and $5.5 \text{ mmol} \cdot \text{L}^{-1}$, $7.6 \text{ mU} \cdot \text{L}^{-1}$, and $90 \text{ ng} \cdot \text{L}^{-1}$, respectively, for the HC2-group.

Fitting the INT_M1 and INT_M2 outputs (dash-dot line in Figure 2.2 C and F) to mean insulinemia data from OGTT-matched I-IVG protocols, reported for the HC1-group and HC2-group (open circles in Figure 2.2 C and F, respectively), yielded the RMSE$_{I_{-IVG}}$ and the estimates of $k_7$ given in Table 2.3 for the former model and in Table 2.4 for the latter.

After feeding the INT_M1 and the INT_M2 with the $k_7$ values obtained from the previous step, the INC(t), G(t) and I(t) responses to a 75 g OGTT were generated and fitted to incretin, glycemia and insulinemia data from OGTT protocols corresponding to each I-IVG, thus estimating the $k_5$, $k_8$, $M$, $\gamma$, $k_e$ and $\beta$ values reported in Table 2.3 for the INT_M1, and the $k_5$, $k_8$, $M$, $\gamma$, $k_{\text{min}}$ and $k_{\text{max}}$ values reported in Table 2.4 for the INT_M2. Eventually, computation of $\varepsilon_1$ (Equation 2.11), $\varepsilon_2$ (Equation 2.12), $Ra_{INC}$ (Equation 2.15), $\lambda$ (Equation 2.17), $k_1$ (Equation 2.20) and $k_2$ (Equation 2.21) yielded the values given in Table 2.5. Figure 2.2 displays the quality of data fit obtained from applying the INT_M1 (dashed line) and the INT_M2 (solid line) to mean OGTT data from the HC1-
group (panels A to C), and the HC2-group (panels D to F). Values of RMSE_{OGTT}
are reported in Table 2.3 for the INT_M1 and in Table 2.4 for the INT_M2.

Figure 2.2. Mean data of plasma incretin (closed triangles in panels A and D), glucose
(closed squares in panels B and E) and insulin (closed circles in panels C and F)
concentrations in response to 75 g oral glucose challenge in the HC1-group (left hand
column) and the HC2-group (right hand column) are matched by the profiles of INC(t),
G(t), and I(t) outputs of the INT_M1 (dashed lines) and the INT_M2 (solid lines) models after free parameter optimization. Open circles in panels C and F are mean plasma insulin concentration data from the HC1- and HC2-group, respectively, measured after the glycemic profiles observed following glucose ingestion (OGTT) were matched by means of an isoglycemic intravenous glucose (I-IVG) infusion (open squares in panels B and E). The dash-dot line in panels C and F describes the best fitting I-IVG insulin output provided by both the INT_M1 and the INT_M2 model.

Figure 2.3 displays the time course of the $G_{empt}$ and $R_{Gi}$ profiles predicted by the INT_M1 (dashed line) and the INT_M2 (solid line) for the HC1-group (panels A and B, respectively) and the HC2-group (panels C and D, respectively). The grey area in panels B and D represents the range of variability of $R_{Gi}$, measured with the multiple tracer, tracer-to-tracer clamp technique during OGTT as reported by Dalla Man et al. [1.35].

Values of the insulin potentiation index, IP%, computed by Equation 1.1 from OGTT and matched I-IVG insulinemia outputs produced by the INT_M1 and INT_M2 models are compared in Table 2.6 with the corresponding IP% values computed from mean insulinemia data of the HC1- and HC2-group.
Figure 2.3. The profiles of the rate of emptying of oral glucose load into the gut, $G_{empt}(t)$, and the rate of glucose absorption from the gut into the mesenteric circulation, $R_{ag}(t)$, for the HC1-group (Panel A and B, respectively) and the HC2-group (Panel C and D, respectively) as predicted by INT_M1 (dashed line) and the INT_M2 (solid line). The grey area in panels B and D represents the range of variability of $R_{ag}$, measured with the multiple tracer, tracer-to-tracer clamp technique during OGTT as reported by Dalla Man et al [1.35].
Table 2.3. Estimates of INT_M1 model parameters in the presence of HGB$_b$ equal to 0.77 mmol·min$^{-1}$.

<table>
<thead>
<tr>
<th></th>
<th>$k_7$ (CV%)</th>
<th>$k_5$ (CV%)</th>
<th>$k_8$ (CV%)</th>
<th>$\gamma$ (CV%)</th>
<th>$M$ (CV%)</th>
<th>$k_e$ (CV%)</th>
<th>$\beta$ (CV%)</th>
<th>RMSE$_{I-IVG}$</th>
<th>RMSE$_{OGTT}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mU·min$^{-1}$·mmol$^{-1}$·L$^{-1}$·mmol$^{-1}$</td>
<td>mU·min$^{-1}$·ng$^{-1}$</td>
<td>mmol·mU$^{-1}$</td>
<td>L$^{-2}$·mU$^{-1}$·min$^{-1}$</td>
<td>min$^{-1}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HC1</td>
<td>0.648 (2.2%)</td>
<td>6.77 (5.3%)</td>
<td>0.027 (4.7%)</td>
<td>0 (-)</td>
<td>0.0024 (24%)</td>
<td>0.0095 (6.9%)</td>
<td>1.0 (2.6%)</td>
<td>2.96</td>
<td>2.21</td>
</tr>
<tr>
<td>HC2</td>
<td>0.158 (2.3%)</td>
<td>8.77 (2.7%)</td>
<td>0.017 (3.4%)</td>
<td>0.058 (4.9%)</td>
<td>0.0034 (5.7%)</td>
<td>0.0155 (1.1%)</td>
<td>1.52 (0.8%)</td>
<td>4.16</td>
<td>3.89</td>
</tr>
</tbody>
</table>

HC1, group of 11 subjects with normal glucose tolerance (NGT) from Muscelli et al. [1.19]; HC2, group of 10 metabolically healthy subjects from Nauck et al. [1.18]; $k_7$, coefficient of appearance of insulin due to glucose, in the absence of incretin effect; $k_5$, gain of the zero-order transfer function between the rate of gastric emptying, $G_{empt}$, and the dynamic plasma incretin component; $k_8$, coefficient of appearance of insulin due to incretin; $\gamma$, shaping factor for derivative control of insulin on glucose; $M$, coefficient accounting for the effects of counter-regulatory factors on liver; $k_e$ and $\beta$, fractional transfer coefficient and dimensionless shape factor of gastric emptying, respectively; CV%, precision of all parameter estimates expressed as percent coefficient of variation; RMSE$_{I-IVG}$, root-mean-square error of I-IVG data fit, RMSE$_{OGTT}$, root-mean-square error of OGTT data fit.
Table 2.4. Estimates of INT_M2 model parameters in the presence of HGBb equal to 0.77 mmol·min\(^{-1}\).

<table>
<thead>
<tr>
<th></th>
<th>(k_7) (CV%)</th>
<th>(k_5) (CV%)</th>
<th>(k_8) (CV%)</th>
<th>(\gamma) (CV%)</th>
<th>(M) (CV%)</th>
<th>(k_{\text{min}}) (CV%)</th>
<th>(k_{\text{max}}) (CV%)</th>
<th>RMSE_IVG</th>
<th>RMSE_OGTT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mU·min(^{-1})·mmol(^{-1})·L(^{-0.3})</td>
<td>ng L(^{-1})·mmol(^{-1})</td>
<td>mU·min(^{-1})·ng(^{-1})</td>
<td>mmol·mU(^{-1})</td>
<td>L(^{-2})·mU(^{-1})·min(^{-3})</td>
<td>min(^{-1})</td>
<td>min(^{-1})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HC1</td>
<td>0.648 (2.2%)</td>
<td>5.78 (3.9%)</td>
<td>0.025 (4.9%)</td>
<td>0.0028 (84%)</td>
<td>0.0071 (5.9%)</td>
<td>0.017 (2.6%)</td>
<td>0.038 (2.7%)</td>
<td>2.96</td>
<td>3.90</td>
</tr>
<tr>
<td>HC2</td>
<td>0.158 (2.3%)</td>
<td>8.82 (2.7%)</td>
<td>0.017 (3.3%)</td>
<td>0.0605 (4.5%)</td>
<td>0.0033 (5.1%)</td>
<td>0.027 (1.8%)</td>
<td>0.038 (1.4%)</td>
<td>4.16</td>
<td>4.07</td>
</tr>
</tbody>
</table>

HC1, group of 11 subjects with normal glucose tolerance (NGT) from Muscelli et al. [1.19]; HC2, group of 10 metabolically healthy subjects from Nauck et al. [1.18]; \(k_7\), coefficient of appearance of insulin due to glucose, in the absence of incretin effect; \(k_5\), gain of the zero-order transfer function between the rate of gastric emptying, \(G_{empt}\), and the dynamic plasma incretin component; \(k_8\), coefficient of appearance of insulin due to incretin; \(\gamma\), shaping factor for derivative control of insulin on glucose; \(M\), coefficient accounting for the effects of counter-regulatory factors on liver; \(k_{\text{min}}\), minimum of the \(k_{\text{empt}}(q_{\text{ins}})\) function; \(k_{\text{max}}\), maximum of \(k_{\text{empt}}(q_{\text{ins}})\) function; CV\%, precision of all parameter estimates expressed as percent coefficient of variation; RMSE\_IVG, root-mean-square error of I-IVG data fit, RMSE\_OGTT, root-mean-square error of OGTT data fit.
Table 2.5. Computed values of INT_M1 and INT_M2 model parameters.

<table>
<thead>
<tr>
<th></th>
<th>ε₁ ( \text{mmol}^{-1} )</th>
<th>ε₂ ( \text{mmol}^{-1} )</th>
<th>Ra_{INCb} ( \text{ng min}^{-1} )</th>
<th>λ ( \text{mU L}^{-1} \text{min}^{-1} )</th>
<th>k₁ ( \text{min}^{-1} )</th>
<th>k₂ ( \text{mmol mU}^{-1} \text{min}^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>INT_M1</td>
<td>HC1</td>
<td>HC2</td>
<td>HC1</td>
<td>HC2</td>
<td>HC1</td>
<td>HC2</td>
</tr>
<tr>
<td>INT_M2</td>
<td>0.040</td>
<td>0.024</td>
<td>0.040</td>
<td>0.024</td>
<td>217</td>
<td>135</td>
</tr>
</tbody>
</table>

HC1, group of 11 subjects with normal glucose tolerance (NGT) from Muscelli et al. [1.19]; HC2, group of 10 metabolically healthy subjects from Nauck et al. [1.18]; ε₁ and ε₂ respectively quantify the rate of decrease and subsequent increase of the gastric emptying function (Equations 2.11 and 2.12, respectively); Ra_{INCb}, basal rate of appearance of incretin in the peripheral circulation (Equation 2.15); λ, effects of additional regulators of I(t) on insulin appearance (Equation 2.17); k₁ and k₂ are coefficients of linear glucose mediated and insulin mediated glucose uptake, respectively (Equations 2.20 and 2.21, respectively).
Table 2.6. Insulin potentiation.

<table>
<thead>
<tr>
<th></th>
<th>HC1</th>
<th>HC2</th>
</tr>
</thead>
<tbody>
<tr>
<td>INT_M1</td>
<td>63.4</td>
<td>81.0</td>
</tr>
<tr>
<td>INT_M2</td>
<td>62.9</td>
<td>81.1</td>
</tr>
<tr>
<td>EXP</td>
<td>63.0</td>
<td>78.1</td>
</tr>
</tbody>
</table>

HC1, group of 11 subjects with normal glucose tolerance (NGT) from Muscelli et al. [1.19]; HC2, group of 10 metabolically healthy subjects from Nauck et al. [1.18]; IP%, insulin potentiation computed by Equation 1.1.

2.6 Discussion

This work yields an improved formulation of a mathematical model previously proposed by Brubaker et al. [1.30] to simulate the glycemia and insulinemia responses to 50 g and 100 g oral glucose administration, by explicitly incorporating the incretin effect. The incretin response, INC(t), is thought to be a direct effect of the rate of emptying of ingested glucose into the gut, G_{empt}(t), through a zero-order transfer function, with k_s gain, between the G_{empt} and the dynamic component of plasma incretin inflow (Equation 2.14). An improved description of intestinal glucose absorption in response to 75 g OGTT, was accomplished here by replacing the previously proposed empirical description,
restricted to 50 g and 100 g glucose challenge [1.30], with either a one-compartment model (M1 in Figure 2.1) and a three-compartment model (M2 in Figure 2.1), thus giving rise to two integrated models denominated INT-M1 and INT-M2, respectively.

Besides the $k_3$, $k_4$, $k_6$, $k_9$, $p$, and $V$ parameters (Table 2.2), which were fixed at numerical values known \textit{a priori} from previously reported measurements, as discussed by Brubaker et al. [1.30], the basal hepatic glucose balance, HGB$_b$, and the four more glucose absorption parameters, $k_{\text{abs}}$, $c$, $b$ and $f$, were fixed at numerical values taken from the literature as described in section 2.1.7. Consequently, seven free parameters ($k_5$, $k_7$, $k_8$, $M$, $\gamma$, $k_c$ and $\beta$ characterize the INT-M1 (Table 2.3), as well as seven are the free parameters ($k_5$, $k_7$, $k_8$, $M$, $\gamma$, $k_{\text{min}}$, and $k_{\text{max}}$) that characterize the INT-M2 (Table 2.4). Among these, the five parameters denominated $k_5$, $k_7$, $k_8$, $M$ and $\gamma$ are the same as those defined “adjustable” by Brubaker et al. [1.30], and presumably manually adjusted in their study for qualitative representation of OGTT responses. The remaining two parameters, i.e. $k_c$ and $\beta$ for the INT-M1 and $k_{\text{min}}$ and $k_{\text{max}}$ for the INT-M2, are free parameters that allow individualization of the rate of ingested glucose absorption. Granted the suitability of all fixed parameters, a novel aspect of the present study was the set-up of a two-step procedure that allows estimation of the free parameters of our INT-M1 and INT-M2 model formulations by fitting to incretin, glycemia and insulinemia data taken from previously published studies.
[1.18,1.19], where glycemic profiles observed in two groups, (HC1 and HC2) of metabolically healthy subjects, during an OGTT, were used to quantify incretin-induced insulin potentiation by comparing the insulin response to OGTT with that obtained from an isoglycemic intravenous glucose (I-IVG) infusion.

According to our two-step estimation procedure, the \( k_7 \) parameter was first estimated from fitting to I-IVG insulinemia data. To this aim the \( G_{empt} \) (and, with it, the dynamic component of plasma incretin inflow in Equation 2.14) was set to zero and the plasma glucose compartment was fed with the glucose profile matched to that measured during OGTT (isoglycemic profile). Under these conditions the alternative formulations (M1 and M2) of glucose absorption do not come into play. This explains why both the INT_M1 and the INT_M2 produce the same \( k_7 \) estimates and related RMSE_{I-IVG} values, as given in Table 2.3 and Table 2.4, respectively. The higher \( k_7 \) estimate of 0.648 \( \text{mU}\cdot\text{min}^{-1}\cdot\text{mmol}^{1.3}\cdot\text{L}^{-0.3} \) for the HC1-group, compared to the estimate of 0.158 \( \text{mU}\cdot\text{min}^{-1}\cdot\text{mmol}^{1.3}\cdot\text{L}^{-0.3} \) for the HC2-group provides quantitative information on an enhanced insulin response to intravenous glucose infusion in the former group. This is consistent with the experimental finding of a higher value of the ratio \( \frac{\text{AUC}_{I-IVG}}{\text{AUC}_{G-IVG}} \) between the area under the curve of I-IVG suprabasal insulin to the area under the curve of the corresponding suprabasal profile of glucose infused intravenously in the HC1-group (ratio of 14.3 mU/mmol from data of Figure 2.2 B and C) compared to that of the HC2-group (ratio of 5.7 mU/mmol from data of Figure 2.2 E and F).
Once the estimated k values were filled into the INT_M1 and the INT_M2, these two models produced a comparable fit to incretin (closed triangles in Figure 2.2 A and D), glycemia (closed squares in Figure 2.2 B and E) and insulinemia (closed circles in Figure 2.2 C and F), as judged from the dashed-line profiles for the INT_M1 and the solid-line profiles for the INT_M2, and the related RMSE$_{OGTT}$ reported in Table 2.3 and Table 2.4, respectively.

Due to their ability to approximate both the OGTT and the matched I-IVG insulin response, the two models yielded estimates of the IP% index of insulin potentiation (Equation 1.1) consistent with the corresponding values computed from measured data in the HC1- and HC2-group (Table 2.6).

The augmented insulin secretion observed after oral glucose load, compared with intravenous glucose infusion, at similar plasma glucose concentration, is attributed to the influence of the incretin effect, most of which is due to the GLP-1 and GIP secretion [1.10-1.12, 1.15, 1.19, 1.34, 1.38]. As the contribution of intestinal hormones to oral glucose-stimulated insulin secretion is relevant, and being the major stimulus to the release of these hormones clearly related to the rate of ingested glucose delivery to the gut, $G_{emp}(t)$, the INC(t) signal described in Equation 2.14 by the product $k_5 \cdot G_{emp}(t)$ was derived from the GLP-1 and GIP responses to OGTT reported in [1.19] for the HC1-group and in [1.18] for the HC2-group, respectively, by hypothesizing an additive effect as described in section 2.3. With this assumption, the closed-triangle data of Figure 2.2 A and D
were obtained. The lower $k_5$ estimates provided by both the INT_M1 (Table 2.3) and the INT_M2 (Table 2.4) for the HC1-group, compared to those provided for the HC2-group, reflect the experimental observation of a lower area under the suprabasal curve of incretin in the HC1-group ($25.6 \cdot 10^3 \text{ng}\cdot\text{L}^{-1}$ over 180 min, Figure 2.2 A) compared to that of the HC2-group ($40.0 \cdot 10^3 \text{ng}\cdot\text{L}^{-1}$ over 180 min, Figure 2.2 D) in response to the 75 g oral glucose challenge. On this basis, the $k_5$ free parameter appears a suitable marker of the amplitude of the incretin response to oral glucose administration. In spite of lower incretin response, the suprabasal insulinemia response of the HC1-group, quantified by the $\text{AUC}_{\text{OGTT}}$ value of $9.03 \cdot 10^3 \text{mU}\cdot\text{L}^{-1}$ over 180 min, is higher than that, $7.70 \cdot 10^3 \text{mU}\cdot\text{L}^{-1}$ over 180 min, observed in the HC2-group (compare Figure 2.2 C and F). This implies the presence, in the HC1-group, of a compensatory increase of the insulin response to the incretin, which is reflected in the increased $k_8$ values estimated in the same group by the INT_M1 (Table 2.3) and the INT_M2 (Table 2.4), compared to those of the HC2-group. In the presence of this compensation, the enhanced insulin potentiation (higher IP\% index; Table 2.6) in the HC2-group, compared to the HC1-group, is mainly explained by the observed reduction of suprabasal insulin response to the OGTT-matched profile of glucose infused intravenously, as marked by a lower $k_7$ in the HC2-group.

In spite of a similar behaviour of the INT_M1 and INT-M2 models in accounting for variations of $k_5$, $k_7$ and $k_8$ estimates between the HC1- and the HC2-
group of healthy subjects, a relevant diversity is seen in that, in the HC1-group, the INT_M1 model estimated a zero value for the $\gamma$ and a value of $M$ 66% lower than that produced by the INT_M2. No model independent information is available to judge the reliability of estimated values for $\gamma$, since the small derivative $\gamma \cdot dI/dt$ term was empirically introduced by Brubaker et al. [1.30] to sharpen the peak in glucose level following entry of glucose into the circulation, and was set equal to 0.06 when $R_{ag} > 0$. This value is comparable with that estimated here for the HC2-group by both the INT_M1 and the INT_M2 (Table 2.3 and Table 2.4). The zero-value estimated for $\gamma$ by the INT_M1 in the HC1 group (Table 2.3), as well as the corresponding very low estimate (with 84% estimation error) provided by the INT_M2 (Table 2.4) might be ascribed to a vanishing role of the $\gamma \cdot dI/dt$ in the presence of a limited glycemia dynamics as observed in the HC1-group compared to the HC2-group (Figure 2B with Figure 2E). Concerning the M parameter, which modulates the bilinear glucose-insulin control on hepatic balance, it is likely that its estimate is affected by the value of $HGB_b$ (Equation 2.13) assumed as fixed to favour model identifiability. Because the value of $10.25 \times 10^{-3}$ mmol·min$^{-1}$·kg$^{-1}$ assumed here for basal hepatic glucose balance per unit BW (yielding $HGB_b$ equal to 0.77 mmol·min$^{-1}$ for a standardized 75 kg individual), differs from the value of $12.22 \times 10^{-3}$ mmol·min$^{-1}$·kg$^{-1}$ (yielding $HGB_b$ equal to 0.92 mmol·min$^{-1}$) previously assumed by Brubaker et al. [1.30], the effect of the latter $HGB_b$ value on the estimates of our free model parameters was tested. Results are given in Table 2.7.
for the INT_M1 and in Table 2.8 for the INT_M2 model. Comparison with Table 2.3 and Table 2.4, respectively, confirms that HGB₈ has a major effect on the M parameter estimation. In particular, the M values of Table 2.7 and Table 2.8 are significantly underestimated with respect to the corresponding values of Table 2.3 and Table 2.4. The zero-value of M produced by the INT_M1 for the HC1 group (Table 2.7) implies a stationary unidirectional HGB flux (equation (2.13)), which is unlikely to occur under normal conditions [1.41, 1.42, 2.8]. For this reason the parameter estimates obtained from our HGB₈ assumption (Tables 2.3 and 2.4) are more physiologically consistent. The lack of quantitative information on the HGB dynamics in humans does not allow assessment of the reliability of absolute values of M when different from zero.

In describing the glucose kinetics by Equation 2.18, a linear non-insulin mediated glucose uptake, k₁·G(t), was assumed here, rather than the k₁·G(t)^1.3 power term originally assumed by Brubaker et al. [1.30]. Under our simplifying assumption, which is consistent with that characterizing minimal models of glucose kinetics [1.27], the estimated values for the k₁ coefficient (Table 2.5) increased in both our HC1 and HC2 groups by about 65%, compared to the k₁ values computed in the presence of the k₁·G(t)^1.3 power term. Practically no effect was observed in the RMSE_{OGTT} of data fit and in the estimates of free model parameters.
Table 2.7. Estimates of INT_M1 model parameters in the presence of HGB₈ equal to 0.92 mmol·min⁻¹.

<table>
<thead>
<tr>
<th></th>
<th>k₇ (CV%)</th>
<th>k₈ (CV%)</th>
<th>k₉ (CV%)</th>
<th>γ (CV%)</th>
<th>M (CV%)</th>
<th>k₉ (CV%)</th>
<th>β (CV%)</th>
<th>RMSEavg</th>
<th>RMSEOGTT</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC1</td>
<td>0.648 (2.2%)</td>
<td>6.86 (5.7%)</td>
<td>0.026 (4.8%)</td>
<td>0 (-)</td>
<td>0 (-)</td>
<td>0.0093 (7.8%)</td>
<td>1.0 (2.9%)</td>
<td>2.96</td>
<td>2.09</td>
</tr>
<tr>
<td>HC2</td>
<td>0.158 (2.3%)</td>
<td>8.85 (1.3%)</td>
<td>0.017 (1.2%)</td>
<td>0.0489 (5.0%)</td>
<td>0.0017 (1.4%)</td>
<td>0.0156 (1.0%)</td>
<td>1.47 (0.9%)</td>
<td>4.16</td>
<td>4.0</td>
</tr>
</tbody>
</table>

See legend of Table 2.3 for meaning of symbols.
Table 2.8. Estimates of INT_M2 model parameters in the presence of HGB<sub>b</sub> equal to 0.92 mmol·min<sup>-1</sup>.

<table>
<thead>
<tr>
<th></th>
<th>( k_7 ) (CV%)</th>
<th>( k_7 ) (CV%)</th>
<th>( k_8 ) (CV%)</th>
<th>( \gamma ) (CV%)</th>
<th>( M ) (CV%)</th>
<th>( k_{max} ) (CV%)</th>
<th>( k_{max} ) (CV%)</th>
<th>RMSE&lt;sub&gt;LSVG&lt;/sub&gt;</th>
<th>RMSE&lt;sub&gt;OGTT&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC1</td>
<td>0.648 (2.2%)</td>
<td>5.95 (3.8%)</td>
<td>0.025 (4.8%)</td>
<td>0.0043 (65%)</td>
<td>0.0043 (9.1%)</td>
<td>0.016 (2.4%)</td>
<td>0.037 (2.5%)</td>
<td>2.96</td>
<td>3.92</td>
</tr>
<tr>
<td>HC2</td>
<td>0.158 (2.3%)</td>
<td>8.93 (2.7%)</td>
<td>0.016 (3.3%)</td>
<td>0.0566 (4.9%)</td>
<td>0.0021 (8.0%)</td>
<td>0.026 (1.8%)</td>
<td>0.037 (1.4%)</td>
<td>4.16</td>
<td>4.14</td>
</tr>
</tbody>
</table>

See legend of Table 2.4 for meaning of symbols.
2.6.1 Monophase vs. multiphase waveshape of gastric emptying of glucose

In conceptual terms, our INT_M1 model differs from the INT_M2 in that the former incorporates the hypothesis of a monophasic waveshape for the rate of gastric emptying of ingested glucose into the gut, \( G_{\text{empt}} \) (Equation 2.1 and dashed line in Figure 2.3 A and C), characterized by the two identifiable parameters, \( k_e \) and \( \beta \) (Table 2.3). Instead, the INT_M2 configuration assumes a three-compartment model of glucose absorption, with a nonlinear gastric emptying function \( k_{\text{empt}} \) (Equation 2.10), that yields a multiphase \( G_{\text{empt}} \) (solid line in Figure 2.3 A and C). Unfortunately, the \( k_{\text{empt}} \) is characterized by four independent parameters (\( b, c, k_{\text{min}} \) and \( k_{\text{max}} \) a couple of which needs to be fixed to favour numerical identifiability of the INT_M2 model. Our choice was, then, to fix the \( b \) and \( c \) values (Table 2.2), which respectively locate the flex points of \( k_{\text{empt}} \) in correspondence of the percentage of the glucose dose, \( D \), for which the emptying function \( k_{\text{empt}} \) (Equation 2.10) decreases and subsequently increases at \( (k_{\text{max}}-k_{\text{min}})/2 \) \([1.35]\). The need of fixing two of the four characteristic parameters of the \( k_{\text{empt}} \) function yields a limitation in the INT_M2 ability to match the data, such that no relevant benefit is seen in the \( \text{RMSE}_{\text{OGTT}} \), compared to the INT_M1 output.
Chapter 3.

Concluding Remarks

An improvement over a mathematical model previously proposed by Brubaker et al. [1.30] to simulate the glycemia and insulinemia responses to an OGTT, was accomplished here by replacing the previously proposed empirical description of glucose absorption, restricted to 50 g and 100 g glucose challenge, with either the M1 and the M2 model configurations depicted in Fig. 1. This new arrangement, gave rise to our INT_M1 and INT_M2 integrated models, respectively, that allow a reliable description of glycemic and insulinemic responses to a clinically consistent 75 g OGTT. Under the hypothesis of an additive effect of GLP-1 and GIP on insulin potentiation, our results showed a substantial equivalence of the two alternative INT_M1 and INT_M2 models in reproducing insulin potentiation as observed by comparing mean OGTT responses to a 75 g oral glucose challenge with matched I-IVG responses, as reported in [1.26] and in [1.24] for two different groups of healthy subjects, here denominated HC1 and HC2, respectively. Especially, the implementation of both the INT_M1 and INT_M2 models in our two-step parameter estimation procedure allowed assessment of $k_5$, $k_7$ and $k_8$ parameters, which appear as suitable markers of the
differences in the incretin, glucose and insulin responses between the two groups. Namely, $k_5$ is a marker of the amplitude of the incretin response to oral glucose administration; $k_8$ is a marker of the insulin response to the incretin, while $k_7$ is a marker of the insulin response irrespective of incretin effect. A higher $k_8$ value estimated by both the INT_M1 and the INT_M2 in the HC1-group, compared to the HC2, indicates the presence, in the former group of an increase of insulin response to incretin, which compensates for a lower incretin response to oral glucose load (lower $k_5$ in the HC1). In the presence of this compensation, the enhanced insulin potentiation (higher IP% index; Table 6) in the HC2-group, compared to the HC1-group, is mainly explained by the observed reduction of suprabasal insulin response to the OGTT-matched profile of glucose infused intravenously, as marked by a lower $k_7$ in the HC2-group. Due to the substantial equivalence in the INT_M1 and INT-M2 behavior, the former model appears as the best compromise between simplicity and predictive capability.

Model implementation in our two-step parameter estimation procedure enhances the possibility of a prospective application for individualization of the incretin effect in a single subject, when his/her data are plugged in.
References


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[1.12] MacDonald PE, Salapatek AM, Wheeler MB. Glucagon-like peptide-1 receptor activation antagonizes voltage-dependent repolarizing K(+) currents in


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