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Mechanism-based population modelling for assessment of L-cell function based on total GLP-1 response following an oral glucose tolerance test

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Abstract GLP-1 is an insulinotropic hormone that synergistically with glucose gives rise to an increased insulin response. Its secretion is increased following a meal and it is thus of interest to describe the secretion of this hormone following an oral glucose tolerance test (OGTT). The aim of this study was to build a mechanism-based population model that describes the time course of total GLP-1 and provides indices for capability of secretion in each subject. The goal was thus to model the secretion of GLP-1, and not its effect on insulin production. Single 75 g doses of glucose were administered orally to a mixed group of subjects ranging from healthy volunteers to patients with type 2 diabetes (T2D). Glucose, insulin, and total GLP-1 concentrations were measured. Prior population data analysis on measurements of glucose and insulin were performed in order to estimate the glucose absorption rate. The individual estimates of absorption rate constants were used in the model for GLP-1 secretion. Estimation of parameters was performed using the FOCE method with interaction implemented in NONMEM VI. The final transit/

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indirect-response model obtained for GLP-1 production following an OGTT included two stimulation components (fast, slow) for the zero-order production rate. The fast stimulation was estimated to be faster than the glucose absorption rate, supporting the presence of a proximal–distal loop for fast secretion from L-cells. The fast component ($st_3 = 8.64{\cdot}10^{-5}$ [mg^{-1}]) was estimated to peak around 25 min after glucose ingestion, whereas the slower component ($st_4 = 26.2{\cdot}10^{-5}$ [mg^{-1}]) was estimated to peak around 100 min. Elimination of total GLP-1 was characterised by a first-order loss. The individual values of the early phase GLP-1 secretion parameter (st_3) were correlated ($r = 0.52$) with the AUC(0–60 min.) for GLP-1. A mechanistic population model was successfully developed to describe total GLP-1 concentrations over time observed after an OGTT. The model provides indices related to different mechanisms of subject abilities to secrete GLP-1. The model provides a good basis to study influence of different demographic factors on these components, presented mainly by indices of the fast- and slow phases of GLP-1 response.

Keywords GLP-1 · L-cells · Oral glucose tolerance test (OGTT) · Indirect response model · NONMEM

Introduction

Type 2 diabetes (T2D) is a result of decreased insulin sensitivity combined with decreased beta-cell function. The beta-cell function is described by the ability of the beta-cells to provide an insulin response to a given glucose load.

One of the main determinants of beta-cell function is the presence of the insulinotropic hormone glucagon-like-peptide 1 (GLP-1) [1, 2] in combination with glucose. More specifically Brandt et al. [2] demonstrated in vivo glucose dependency of the action of postprandial physiological concentrations of GLP-1 in healthy subjects over the plasma glucose range of 5–10 mM.

GLP-1 is a gut derived peptide secreted from intestinal L-cells [3] and circulating levels increase after a meal or an oral glucose load [4, 5]. It is derived from a transcription product of the proglucagon gene and the active molecule is identified as GLP-1 (7–36). Once in the circulation it has a very short half-life estimated to be around 2–3 min in healthy volunteers [4].

The GLP-1 response in terms of area under the curve from 0 to 240 min. after the start of the meal is significantly decreased in most patients with type 2 diabetes [6]. Combined with the finding that the short half-life of GLP-1 does not seem to differ in healthy volunteers and patients with T2D [1], this suggests that the decreased GLP-1 response observed in patients with T2 diabetes is due to a lower post-prandial secretion. This also seems to be the case comparing patients with impaired glucose tolerance (IGT) and healthy volunteers [5]. In general we believe that analysis of the GLP-1 response observed after an OGTT would be valuable in understanding the mechanisms underlying the post-prandial secretion profile.

The overall aim of this study was to develop a mechanism-based population model providing descriptive indices of the observed GLP-1 secretion following an

OGTT. The goal was thus not to model the GLP-1 effect on insulin secretion, but rather to build a model providing indices for capability of GLP-1 secretion. Based on the mechanisms of action, we propose to model the stimulation of GLP-1, using an indirect response model [7]. Compared to earlier non-compartmental analysis (as in [8]) of the GLP-1 secretion profiles observed after an OGTT, a compartmental population model approach takes into account variability in measurements and time (compartmental) and variability between subjects (population). This kind of model further provides a good basis for future inclusion of covariates (such as demographic factors) on obtained model parameters.

Methods

Study participants

The data applied in this study is a subset of the dataset originally described in [9]. In this study available plasma GLP-1 profiles obtained after an oral glucose load are included. Only full profiles were included and seven profiles were removed because of erratic behaviour inconsistent with basic physiology and the dynamics of the rest of the population. The cleaned dataset applied here thus consisted of samples taken from 135 individuals distributed as presented in Table 1. The classification of individuals was categorized according to concentrations of plasma glucose (FPG) fasting and 2 h after glucose ingestion ($OGTT_{120}$) measured in mmol/L. The classification criteria, agreed with the ones described in [10]. The study was approved by the Ethical Committee of Copenhagen and was in accordance with the principles of the Declaration of Helsinki.

Study conditions

All participants underwent a standardized and extended 75-g frequently sampled OGTT. After a 12-h overnight fast, venous blood samples were drawn in duplicate at −30, −10, 0 before the glucose intake and then at 10, 20, 30, 40, 50, 60, 75, 90, 105, 120, 140, 160, 180, 210, 240. Plasma glucose and serum insulin were measured. The plasma glucose concentration was analyzed by a glucose oxidase method (Granutest; Merck, Darmstadt, Germany). Serum insulin was determined by

Table 1 Mean and standard deviation (SD) of demographics of study subjects

Subjects	Normal	IFG-IGT-T2D	Total
Number	117	18	135
Age [yr]	41.8 (11.4)	45.6 (12.7)	42.3 (11.6)
Fasting plasma glucose [mg dl^{-1}]	93.0 (8.1)	109.8 (13)	95.3 (10.5)
Fasting plasma insulin [pmol l^{-1}]	5.43 (3.1)	11.66 (8.4)	6.26 (4.6)
Fasting plasma GLP-1(total) [pmol l^{-1}]	5.35 (3.3)	4.61 (2.6)	5.26 (3.2)

IFG Impaired fasting glucose, *IGT* Impaired glucose tolerance, *T2D* Type 2 diabetics

enzyme-linked immunoadsorbent assay with a narrow specificity excluding des (31, 32)-proinsulin and intact proinsulin (DAKO Diagnostics, Ely, UK) [11].

Fasting plasma GLP-1 were analysed in duplicate and at single measurements post glucose load at time points 10, 20, 30, 40, 60, 90, 120, 180, and 240 min. All blood samples for GLP-1 analysis were kept on ice, and the protease inhibitor aprotinin (Novo Nordisk, Denmark) was added in a concentration of 0.08 mg/ml blood. The GLP-1 concentrations were measured after extraction of plasma with 70% ethanol (vol/vol). The plasma concentrations of GLP-1 were measured [12] using standards of synthetic GLP-1 7–36 amide using antiserum code no. 89390, which is specific for the amidated C-terminus of GLP-1 and therefore mainly reacts with GLP-1 derived from the intestine. The results of the assay reflect the rate of secretion of GLP-1 because the assay measures the sum of intact GLP-1 and the primary metabolite, GLP-1 9–36 amide, into which GLP-1 is rapidly converted [13]. The assay sensitivity was below 1 pmol/l, intra-assay coefficient of variation below 0.06 at 20 pmol/l, and recovery of standard added to plasma before extraction was 100% when corrected for losses inherent in the plasma extraction procedure. Very few samples were under the LLOQ, and these were not included in analysis.

Non-compartmental analysis

The individual incremental areas under the curve for GLP-1 were calculated using a linear up/linear down trapezoidal method. Peak AUCs identified in the report as $AUCP_{GLP\text{-}1}$ were calculated as incremental AUCs up to 60 min. The software S-plus was used for this part of the analysis.

Compartmental population modelling

For preliminary analysis, the absorption rate constant (k_a) of glucose was obtained from glucose and insulin data by applying the model presented by Lima et al. [14], using two compartments for description of absorption rate according to Eq. (3) and (4). This was done in order not to bias the estimation of this parameter towards the fitting of GLP-1.

Baseline GLP-1 values were calculated as the average from pre-dose samples for each individual. Considering the fact that the inclusion of these baseline values as either fixed or estimated can influence the bias of other parameters [15], we implemented these values as either fixed, fixed with a variance, or estimated. In general the GLP-1 data was modelled using a population model build in NONMEM VI using the FOCE Inter method. Model selection was based on individual/population predicted profiles, variance and independence of residuals, and obtained objective function value (OFV), and inspection of visual predictive check (VPC).

Structural model

The final structural models for glucose/insulin and secretion of GLP-1 are presented in Fig. 1. The glucose/insulin model was applied in order to obtain estimates of glucose absorption rate. The model for the GLP-1 secretion reflects an indirect

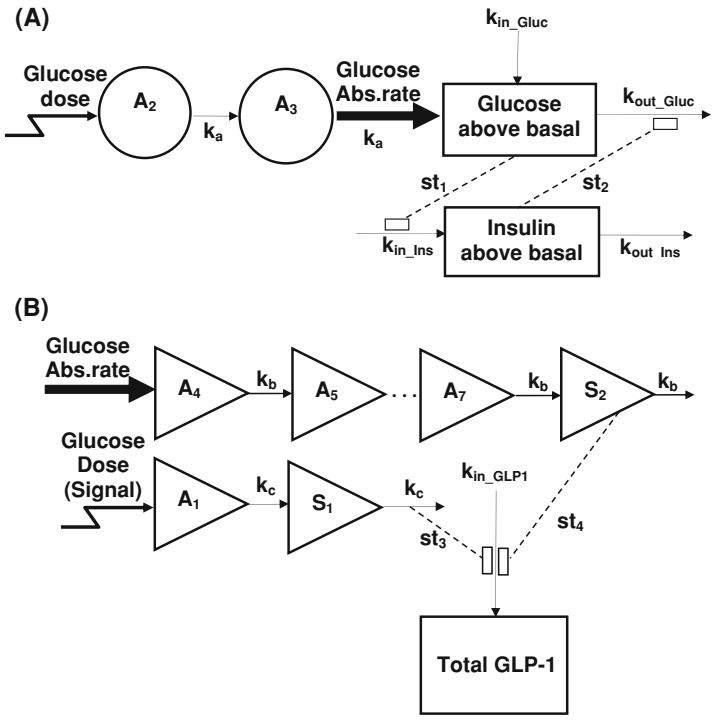

Fig. 1 **a** Diagram of glucose/insulin model for estimation of glucose absorption rate constant, **b** GLP-1 secretion model. Absorption rate for glucose is identical to that estimated in the glucose/insulin model. Symbols are defined in Table 2

response model with zero-order input and first-order loss. The zero-order input was found to be stimulated by two mechanisms differentiated by time of onset. The first part was estimated to be faster than the absorption of glucose and caused a peak in the GLP-1 concentration around 40 min as also identified in [16]. The ingestion signal was included as being proportional to the glucose dose size as:

$$\frac{dA_1}{dt} = -k_c \cdot A_1, \quad A_1(0) = Dose \tag{1}$$

$$\frac{dS_1}{dt} = k_c \cdot A_1 - k_c \cdot S_1, \quad S_1(0) = 0 \tag{2}$$

where $1/k_c$ [min] determines the length of the signal caused by the intake of the amount of glucose, defined by *D*ose. The A_1 and S_1 define the first and second transit compartments in the early response signal originating from ingestion of glucose. The second part was related to a delayed version of the absorption of glucose in gut. The delay was implemented with the use of transit compartments.

The optimal number of transit compartments for description of the delay was determined based on an explicit solution [17] together with the obtained OF Vs.

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