PLOS | ONE

RESEARCH ARTICLE

A Mechanistic Model of Intermittent Gastric Emptying and Glucose-Insulin Dynamics following a Meal Containing Milk Components

Priska Stahel[1☯*], John P. Cant[1☯], Jayden A. R. MacPherson[2‡], Harma Berends[3‡], Michael A. Steele[2‡]

1 Department of Animal Biosciences, University of Guelph, Guelph, Ontario, Canada, **2** Department of Agriculture, Food and Nutritional Services, University of Alberta, Edmonton, Alberta, Canada, **3** Trouw Nutrition R&D, Boxmeer, North Brabant, the Netherlands

☯ These authors contributed equally to this work.
‡ These authors also contributed equally to this work.
* pstahel@uoguelph.ca

Citation: Stahel P, Cant JP, MacPherson JAR, Berends H, Steele MA (2016) A Mechanistic Model of Intermittent Gastric Emptying and Glucose-Insulin Dynamics following a Meal Containing Milk Components. PLoS ONE 11(6): e0156443. doi:10.1371/journal.pone.0156443

Editor: Daniel E Crocker, Sonoma State University, UNITED STATES

Received: January 22, 2016

Accepted: May 13, 2016

Published: June 2, 2016

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: The co-author Harma Berends is employed by Trouw Nutrition R&D, Boxmeer, The Netherlands, but this does not alter the authors' adherence to PLOS ONE policies on sharing data and materials. Trouw Nutrition R&D provided support in the form of salary for author Harma Berends, but did not have any additional role in the model design and evaluation, decision to publish, or final preparation of the manuscript. The specific role of this author is articulated in the 'author contributions' section.

Abstract

To support decision-making around diet selection choices to manage glycemia following a meal, a novel mechanistic model of intermittent gastric emptying and plasma glucose-insulin dynamics was developed. Model development was guided by postprandial timecourses of plasma glucose, insulin and the gastric emptying marker acetaminophen in infant calves fed meals of 2 or 4 L milk replacer. Assigning a fast, slow or zero first-order gastric emptying rate to each interval between plasma samples fit acetaminophen curves with prediction errors equal to 9% of the mean observed acetaminophen concentration. Those gastric emptying parameters were applied to glucose appearance in conjunction with minimal models of glucose disposal and insulin dynamics to describe postprandial glycemia and insulinemia. The final model contains 20 parameters, 8 of which can be obtained by direct measurement and 12 by fitting to observations. The minimal model of intestinal glucose delivery contains 2 gastric emptying parameters and a third parameter describing the time lag between emptying and appearance of glucose in plasma. Sensitivity analysis of the aggregate model revealed that gastric emptying rate influences area under the plasma insulin curve but has little effect on area under the plasma glucose curve. This result indicates that pancreatic responsiveness is influenced by gastric emptying rate as a consequence of the quasi-exponential relationship between plasma glucose concentration and pancreatic insulin release. The fitted aggregate model was able to reproduce the multiple postprandial rises and falls in plasma glucose concentration observed in calves consuming a normal-sized meal containing milk components.

Competing Interests: The co-author Harma Berends is employed by Trouw Nutrition R&D, Boxmeer, The Netherlands, but this does not alter the authors' adherence to PLOS ONE policies on sharing data and materials.

Introduction

The mathematical simulation of glucose-insulin dynamics in response to a meal is of great value for decision support related to management of plasma glucose in animals under our care, including domestic species and human patients. The classification of foods according to their effect on the incremental area under the curve of plasma glucose concentrations after ingestion, the so-called glycemic index, was developed to assist in glycemia management [1]. The glycemic response is not just a characteristic of foods, however. Upon consumption of a meal, the ability to dispose of the absorbed glucose, and thus minimize postprandial hyperglycemia and its potentially negative consequences, is dependent on the subject's pancreatic responsiveness, insulin sensitivity and glucose effectiveness. Absorbed glucose has a varying ability to stimulate pancreatic insulin secretion across individuals, with type 1 diabetes being an extreme case in which insulin is only minimally secreted or not at all. The circulating insulin acts on the liver, muscle and adipose tissues to decrease hepatic glucose production and increase peripheral glucose disposal, respectively. In addition, glucose can stimulate its own disposal via a mass-action effect on influx into various tissues. Impairments in any of these three factors of pancreatic responsiveness, insulin sensitivity or glucose effectiveness can exacerbate postprandial hyperglycemia, a hallmark of metabolic syndrome and type 2 diabetes. The minimal glucose and insulin models of Bergman *et al.* [2] and Toffolo *et al.* [3] describe all three of these contributions to glucose disposal following an intravenous glucose dose.

Aside from the prominent role of insulin, appearance of glucose in plasma following ingestion of a carbohydrate laden meal is dependent on the rate of gastric emptying. In the early phase of meal consumption, the proximal gastric wall distends and relaxes to accommodate the increased volume. This is quickly followed by tonic contractions of the proximal stomach to push contents towards the distal end where large particles are degraded, aided by the action of peristaltic contractions, and from which small particles and liquids can be pushed into the duodenum through the pyloric sphincter [4]. Liquids display exponential gastric emptying proportional to the gastric volume and without an initial lag, while solids show biphasic gastric emptying with a lag [4]. The intestine responds to early sampling of stomach contents following emptying by altering gut peptide secretion, dependent on the characteristics of the meal. This altered gut peptide profile influences neuro-hormonal control of gastrointestinal function and gastric emptying. Overall, postprandial gastric emptying is a function of meal composition, volume, osmolality and caloric load [4,5].

Equations to simulate gastric emptying have been incorporated into models to interpret plasma and glucose curves following an oral glucose tolerance test [6,7] but we are aware of only one effort to include gastric emptying equations in the simulation of glycemic responses to a normal-sized meal [8,9]. To simulate the pattern of plasma glucose concentrations following a meal, Dalla Man *et al.* [9] combined a gastric emptying/absorption model of 9 parameters with a glucose-insulin model of 26 parameters, where the 4 components of gastric emptying/absorption, glucose utilization, endogenous glucose production, and pancreatic insulin secretion were each fitted separately from tracer flux and plasma concentration data. Part of the difficulty with modelling gastric emptying is that outflow from the stomach is intermittent, which produces an erratic timecourse. We have previously used models of 2 to 4 parameters to describe the intermittent appearance of acetaminophen (Ac) in plasma following an oral dose [10]. Acetaminophen is very slowly absorbed from the stomach but rapidly absorbed in the proximal small intestine [11] so its appearance in plasma can be used to estimate gastric emptying rate [10].

In this paper, we present the development and evaluation of a novel mechanistic model that incorporates glucose-insulin dynamics of MINMOD [12] with an intermittent gastric emptying model [10] to predict glycemic responses to a milk-based meal in infant cattle. Prior to development of the ruminant habit, the abomasum of pre-weaned calves functions like the glandular stomach of monogastric animals [13] The carbohydrate in milk is the liquid-associated disaccharide lactose, made up of galactose and glucose moieties. It is hydrolyzed by intestinal lactase (EC 3.2.1.108) and approximately 90% of the absorbed galactose is converted to glycogen in the liver [14,15] while approximately 90% of the absorbed glucose enters the peripheral circulation as free glucose [7,9]. We used the aggregate model to evaluate the importance of gastric emptying to postprandial glycemia, relative to the parameters of insulin release and glucose disposal.

Model Structure

Database

Four Holstein-Friesian female calves, housed individually in wheat-straw bedded hutches, at the Trouw Nutrition Ruminant Research facility (Boxmeer, The Netherlands) were maintained on either 4 or 8L of milk replacer (150 g/L dry matter; 24% crude protein, 18% crude fat, and 45.2% lactose on a dry basis; Trouw Nutrition, Deventer, The Netherlands) given twice daily from day 8 to 7 weeks of age. At 4 and 7 weeks of age, calves were provided their morning meal of milk replacer containing 150 mg/kg $BW^{0.75}$ Ac as a gastric emptying marker. Blood samples were taken from a jugular vein catheter at -30, 30, 60, 90, 120, 150, 180, 210, 240, 300, 360 and 420 minutes relative to the meal and plasma was analyzed for cAc_P, cGl_P and cIn_P. The -30-min time-point was used as the 0-min time-point in the model (S1 file). The meals of 2 and 4 L contained 136 and 271 g glucose, respectively, producing a range of 1.8 to 5.1 g/kg BW. Postprandial timecourses of plasma concentrations of acetaminophen (cAc_P), glucose (cGl_P) and insulin (cIn_P) in calves at 4 and 7 weeks of age from MacPherson *et al.* [16] were used to guide model development. Procedures complied with the Dutch Law on Experimental Animals, and the ETS123 (Council of Europe 1985 and the 86/609/EEC Directive) and were approved by the Animal Care and Use Committee from Utrecht University.

The animal datasets are shown in Fig 1. The timecourses of cGl_P and cIn_P do not all exhibit the ideal behaviour of an oral glucose tolerance test, where there is typically a rapid rise to a peak followed by a sustained decline back to baseline. Rather, the concentrations tend to fluctuate up and down throughout the 420 min, in delayed synchrony with changes in cAc_P that reflect gastric emptying rates, and may even sink below baseline concentrations prior to the meal. These erratic behaviours must be accommodated in the simulation model. All model parameters and descriptions are listed in Table 1.

Gastric Emptying

To simulate gastric emptying of Ac in horses, Cant *et al.* [10] assumed that flow of digesta out of the stomach was either on or off during intervals between successive blood samples and multiplied the first-order rate constant for gastric emptying (k_{SP}) by a Z-value of either 1 or 0 to indicate whether gastric emptying was occurring ($\Delta cAc_{P,i} > 0$ in sampling interval i) or not ($\Delta cAc_{P,i} \leq 0$), respectively. We used the same approach, where initial mass of Ac in the stomach (iAc_S = initial Ac_S) is given by the dose of Ac administered with the meal, and the rate of disappearance to blood follows first order kinetics (Fig 2):

$$\frac{dAc_S}{dt} = -k_{SP} \times Ac_S \times Z. \tag{1}$$

Plasma Ac (Ac_P) arises from gastric emptying and disappears according to the first-order

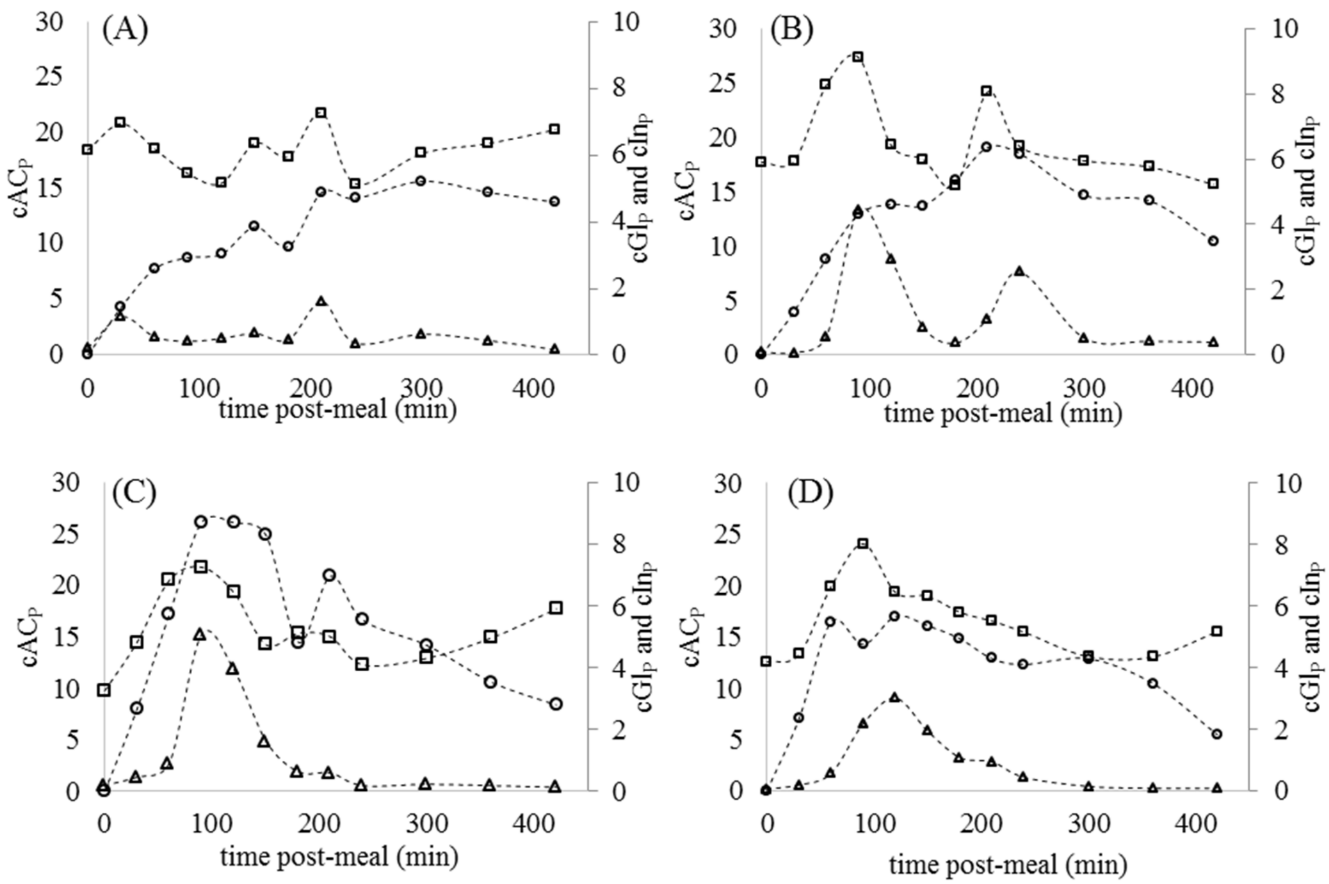

Fig 1. Observed postprandial plasma glucose (cGI$_P$—square, mM), insulin (cIn$_P$–triangle, ng/ml) and acetaminophen (cAC$_P$–circle, mg/ml) relative to meal intake at time = 0 minutes for four representative data sets. (A) Animal 1. (B) Animal 2. (C) Animal 3. (D) Animal 4.

doi:10.1371/journal.pone.0156443.g001

elimination constant ($k_{Ac,UAc}$):

$$\frac{dAc_P}{dt} = k_{SP} \times Ac_S \times Z - k_{Ac,UAc} \times Ac_P. \quad (2)$$

Volume of distribution of Ac_P was fixed at 0.9 L/kg BW [10], so that

$$cAc_P = \frac{Ac_P}{0.9BW}. \quad (3)$$

Best-fit parameters of analytical solutions of the gastric emptying equations were estimated with the Solver function of Microsoft® Office Excel® 2007 to minimize residual sums of squares between predicted and observed cAc_P. Curve fits were evaluated based on the root mean square prediction error (rMSPE) as a percentage of the mean, calculated as:

$$rMSPE\% = \sqrt{\frac{\sum_{i=1}^{n}(pred_i - obs_i)^2}{n}} \Bigg/ \frac{\sum_{i=1}^{n} obs_i}{n}, \quad (4)$$

where $pred_i$ is the i-th prediction, obs_i is the i-th observation, and n is the number of observations.

Table 1. Variable descriptions and reference animal parameter values.

	Units	Ref animal	Description
Animal			
BW	kg	60	Body weight
iAc_S	mg	3234	Dose of Ac administered orally (150 mg/kg $BW^{0.75}$)
iAc_P	mg	0	Basal plasma Ac mass prior to Ac bolus
iGl_S	mmol	396	Dose of glucose present in meal
iGl_P	mmol	90	Basal plasma glucose mass prior to meal
iIn_P	mg	5.92	Basal plasma insulin mass prior to meal
iIs	mg	0.393	Basal insulin signal mass prior to meal
Ac_S	mg	Variable	Stomach Ac mass
Ac_P	mg	Variable	Plasma Ac mass
cAc_P	mg L^{-1}	Variable	Plasma Ac concentration
Gl_S	mmol	Variable	Stomach glucose mass
Gl_P	mmol	Variable	Plasma glucose mass
cGl_P	mmol L^{-1}	Variable	Plasma glucose concentration
In_P	μg	Variable	Plasma insulin mass
cIn_P	μg L^{-1}	Variable	Plasma insulin concentration
Is	μg L^{-1}	Variable	Insulin signal
Acetaminophen			
$k_{SP,2}$	min^{-1}	0.0015	First-order, slow gastric emptying rate constant
$k_{SP,3}$	min^{-1}	0.003	First-order, fast gastric emptying rate constant
$k_{Ac,UAc}$	min^{-1}	0.0022	First-order Ac utilization rate constant
Z	unitless	2,1,2,2,1,0,0,1,1,1,0,0,0,0	Z = 2,1, or 0 when gastric emptying is fast, slow, or not occurring, respectively
Glucose			
$k_{Gl,UGl}$	L min^{-1}	8.7e-7	First-order, glucose-dependent glucose utilization rate constant
$k_{Is,UGl}$	L^2 $μg^{-1}$ min^{-1}	0.0757	First-order, insulin-dependent glucose utilization rate constant
$iPGl_{end}$	mmol min^{-1}	0.178	Initial rate of endogenous glucose production
$T_{lag,SP}$	min	15	Absorption lag time from stomach to plasma
Insulin			
$K_{Gl,Pin}$	mmol L^{-1}	8.8	Glucose-dependent insulin secretion Michaelis constant
$k_{In,UIn}$	L min^{-1}	0.7	First-order insulin utilization rate constant
V_{Pin}	μg min^{-1}	10	Maximal rate of insulin secretion
exp_{Pin}	unitless	9	Hill coefficient for glucose-dependent insulin secretion
$T_{lag,Is}$	min	16	Signaling lag time from In to Is

doi:10.1371/journal.pone.0156443.t001

The model predicted cAc_P with an average rMSPE across all 4 calves of 12% of the mean observed cAc_P. However, the model did not capture the nuances of apparently intermediate outflows between k_{SP} and 0 (arrows in Fig 3). Such intermediate flows were considered important to reproduce because of the changes in plasma glucose and insulin concentrations with which they were associated (Fig 1). An intermediate rate of Ac_P appearance indicates reduced or discontinuous outflow from the stomach during the sampling interval. This intermediate flow was accommodated with a second, non-zero k_{SP} value based on the slope ($\Delta cAc_{P,}$) between successive time points (i). Thus, $k_{SP} = 0$ when $\Delta cAc_{P,i} < -0.05$ mg L^{-1} min^{-1}, $k_{SP,2}$ when -0.05 mg L^{-1} $min^{-1} \leq \Delta cAc_{P,i} \leq 0.05$ mg L^{-1} min^{-1}, and $k_{SP,3}$ when $\Delta cAc_{P,i} > 0.05$ mg L^{-1} min^{-1}. The threshold value of 0.05 mg L^{-1} min^{-1} was chosen to minimize rMSPE. The parameter Z in eqs 1 and 2 was replaced with an array of Z-values equal to 0, 1 or 2 to denote $k_{SP} = 0$,

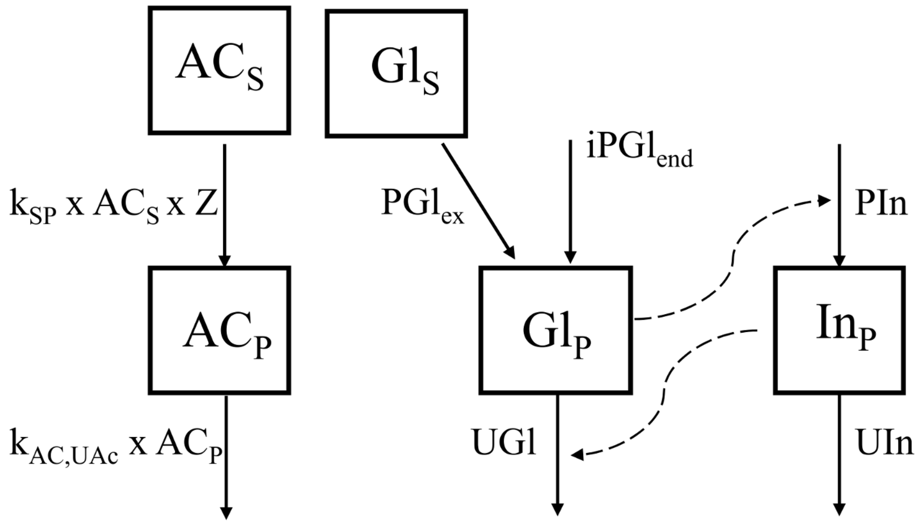

Fig 2. Schematic representation of a mechanistic model simulating intermittent gastric emptying and glucose-insulin dynamics. Solid arrows represent mass fluxes (see Table 1 for parameter descriptions); dashed lines represent stimulatory effects; the dotted line represents inhibitory effect; boxes represent state variables; Ac_S = stomach acetaminophen (mg), Ac_P = plasma acetaminophen (mg), Gl_S = stomach glucose (mmol), Gl_P = plasma glucose (mmol), In_P = plasma insulin (μg), k_{SP} = gastric emptying rate constant (min^{-1}), $k_{Ac,UAc}$ = first-order acetaminophen utilization rate constant (min^{-1}), PGl_{ex} = rate of exogenous glucose appearance ($mmol\ min^{-1}$),), $iPGl_{end}$ = rate of endogenous glucose appearance ($mmol\ min^{-1}$), PIn = rate of pancreatic insulin release (μg/min), UIn = rate of insulin utilization (μg/min).

doi:10.1371/journal.pone.0156443.g002

$k_{SP,2}$ or $k_{SP,3}$, respectively, for each sampling interval (Fig 3). The prediction errors decreased from 12 to 9%, on average, when an intermediate gastric outflow was allowed (Fig 3).

Glucose-Insulin Dynamics

Plasma glucose dynamics (Fig 2) are due to exogenous appearance (PGl_{ex}) and endogenous production (PGl_{end}) and utilization of glucose (UGl), so that

$$\frac{dGl_P}{dt} = PGl_{ex} + PGl_{end} - UGl. \tag{5}$$

Gastric emptying rates $k_{SP,2}$ and $k_{SP,3}$ were used to represent emptying of carbohydrate in the meal from the stomach to the small intestine, as

$$\frac{dGl_S}{dt} = -k_{SP} \times Gl_S. \tag{6}$$

Acetaminophen appearance in plasma during the first 30 min after the meal was typically as rapid as its appearance in the next 30 min (Fig 1), indicating uninterrupted gastric emptying of the meal during the first 60 min. However, plasma glucose concentrations did not typically increase until after the 30-min sample (Fig 1), which could be due to retention of lactose in the stomach or an additional time period for intestinal carbohydrate hydrolysis and absorption. To simulate the delay between the deliveries of acetaminophen and glucose into the plasma, we assigned an absorption time-lag of $T_{lag,SP}$ minutes to the gastric emptying, so that

$$PGl_{ex}(t) = k_{SP} \times Gl_S(t - T_{lag,SP}). \tag{7}$$

To simulate basal cGl_P before the meal, when $PGl_{ex} = 0$, consideration was given to PGl_{end}. It is known that PGl_{end} decreases as PGl_{ex} increases following a meal, due in part to the effects

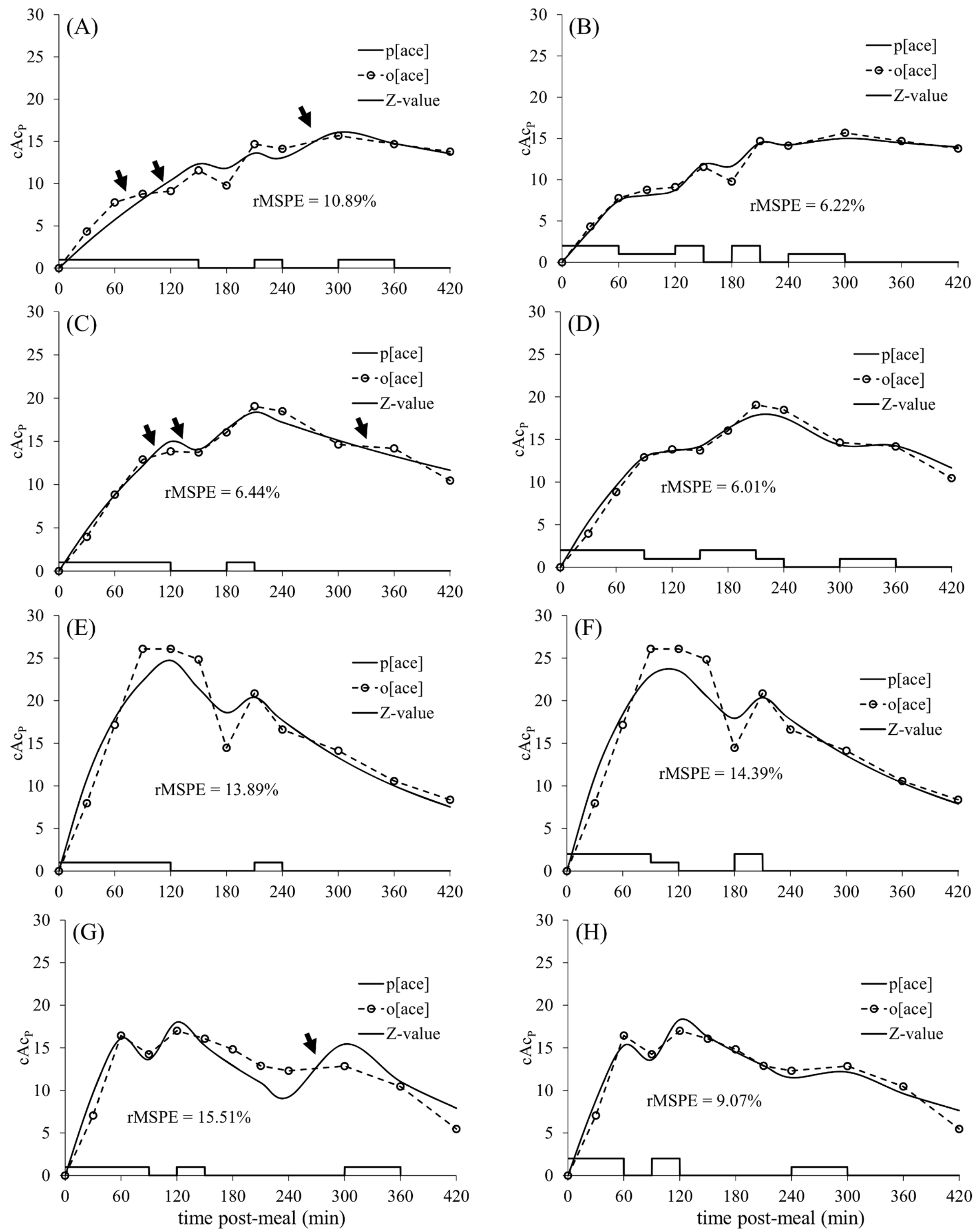

Fig 3. Observed (circles) and predicted (solid lines) post-prandial concentrations of plasma acetaminophen (cAc_P; mg/ml) using 1 (A,C,E,G) or 2 (B,D,F,H) non-zero k_{SP} values for four test datasets. (A,B) Animal 1. (C,D) Animal 2. (E,F) Animal 3. (G, H) Animal 4. Arrows indicate where intermediate gastric outflow rates are apparent.

doi:10.1371/journal.pone.0156443.g003

of insulin on gluconeogenesis and glycogenolysis. However, the PGl_{end}-suppressing effects of insulin on cGl_P are indistinguishable from the UGl-stimulating effects of insulin on cGl_P so we chose to represent insulin effects on cGl_P via UGl, according to the established equations of the Bergman *et al.* [2] minimal model, and not via PGl_{end}. Thus, PGl_{end} is maintained at a constant, zero-order flux equal to an initial PGl_{end} ($iPGl_{end}$), and UGl is a function of cGl_P and the insulin signal (Is) according to a glucose effectiveness constant ($k_{Gl,UGl}$) and an insulin sensitivity constant ($k_{Is,UGl}$):

$$UGl = k_{Gl,UGl} \times cGl_P + k_{Is,UGl} \times Is \times cGl_P. \tag{8}$$

The Is in MINMOD is a first-order delay of cIn_P [12]. This approach is adequate when cIn_P exhibits a single peak in the timecourse, but if cIn_P rises and falls multiple times following a meal, due to intermittent gastric emptying, the first-order delay removes much of the temporal variation in Is. To retain this temporal variation in Is in the current model, we represented the delay as a time lag of $T_{lag,IS}$ min, so that

$$Is(t) = cIn_P(t - T_{lag,IS}). \tag{9}$$

Prior to $T_{lag,IS}$, Is(t) is given an initial value (iIs) equal to the baseline cIn_P.

The differential equation for In (Fig 2) contains the difference between insulin production (PIn) and utilization (UIn):

$$\frac{dIn_P}{dt} = PIn - UIn. \tag{10}$$

In MINMOD, PIn is a linear function of cGl_P above a certain threshold [12]. This structure accommodates, with 2 parameters, the quasi-exponential relation between cGl_P and pancreatic insulin release at the lower end of its sigmoidal relationship [17] but it introduces a breakpoint around the threshold cGl_P value that causes the first derivative to be discontinuous, which we considered undesirable for continuous simulations that may cross this threshold several times in one run. We chose to simulate sigmoidal kinetics of PIn relative to cGl_P continuously with the Hill equation of 3 parameters (V_{PIn}, $K_{Gl,PIn}$ and exp_{PIn}):

$$PIn = \frac{V_{PIn}}{1 + (K_{Gl,PIn}/cGl_P)^{exp_{PIn}}}. \tag{11}$$

UIn remains, as in MINMOD, a mass-action effect of cIn_P according to the first-order rate constant $k_{In,UIn}$:

$$UIn = k_{In,UIn} \times cIn_P \tag{12}$$

Concentrations of Gl_P and In_P are calculated assuming a volume of distribution equal to 0.251 L/kg BW, previously estimated in calves [18]:

$$cGl_P = \frac{Gl_P}{0.251BW} \tag{13}$$

and

$$cIn_P = \frac{In_P}{0.251BW} \tag{14}$$

Behaviour and Sensitivity Analyses

Differential equations of the model were written in ACSLX (Aegis Technologies Group, Inc., Orlando, USA) and solved with a 4th-order Runge-Kutta algorithm using an integration step size of 0.002 min. The final model contains 9 variables and 20 parameters (Table 1, S2 file), 8 of which can be obtained by direct measurement (BW, Z, iAc_S, iAc_P, iGl_S, iGl_P, iIn_P and iIs, where i represents the initial value) and 12 by fitting to observations ($k_{SP,2}$, $k_{SP,3}$, $k_{Ac,UAc}$, $k_{Gl,UGl}$, $k_{Is,UGl}$, $iPGl_{end}$, $T_{lag,SP}$, $K_{Gl,PIn}$, $k_{In,UIn}$, V_{PIn}, exp_{PIn} and $T_{lag,IS}$). The model reproduces the multiple rises and falls in cGl_P and cIn_P that occur in calves during the 420 min following consumption of a normal-sized meal (Figs 4–6). Depending on parameter values, predicted cGl_P and cIn_P can fall below their respective baseline values prior to the meal, which is an important behaviour to reproduce as it is not uncommon (Fig 1). Values of the parameters can also affect the timing and degree of the cGl_P response to changes in gastric emptying and cIn_P. In order to understand which characteristics of the postprandial glycemic response are affected by each of the parameters, they were each perturbed above and below reference values to an extent that allowed changes in the cGl_P and cIn_P curves to be examined. Reference values (Table 1) were set for a 60-kg calf consuming an Ac dose of 3234 mg and carbohydrate load of 396 mmol hexose-equivalents, representing a 2-L meal of milk replacer. The array of Z-values indicating fast, slow or zero gastric emptying was set to a typical pattern of fast initial emptying followed by intermittent, slow gushes. It was assumed that digestibility of lactose was 100% [19], 10% of intestinal galactose entered the circulation as free glucose [14,15] and 10% of intestinal glucose was removed by the splanchnic bed during absorption [7,9]. The remaining parameters of glucose-insulin dynamics were set to generate typical patterns of Ac, Gl and In appearance in plasma over the course of 420 min. Parameter assignments were subject to the constraint that differential eqs 5 and 10 equal zero at t = 0, so that the predicted non-steady, post-prandial state arises from a steady, pre-prandial state. Thus, when V_{In}, $K_{Gl,PIn}$, or exp_{In} were perturbed in the sensitivity analysis, $k_{In,UIn}$ was set to

$$k_{In,UIn} = \frac{V_{PIn}}{\frac{iIn_P}{0.251BW}\left[1 + \left(\frac{K_{Gl,PIn}}{{}^{i}Gl_P/_{0.251}BW}\right)^{exp_{PIn}}\right]}, \tag{15}$$

according to the steady-state constraint and eqs 10 to 14. Likewise, when $k_{Gl,UGl}$ or $k_{Is,UGl}$ were

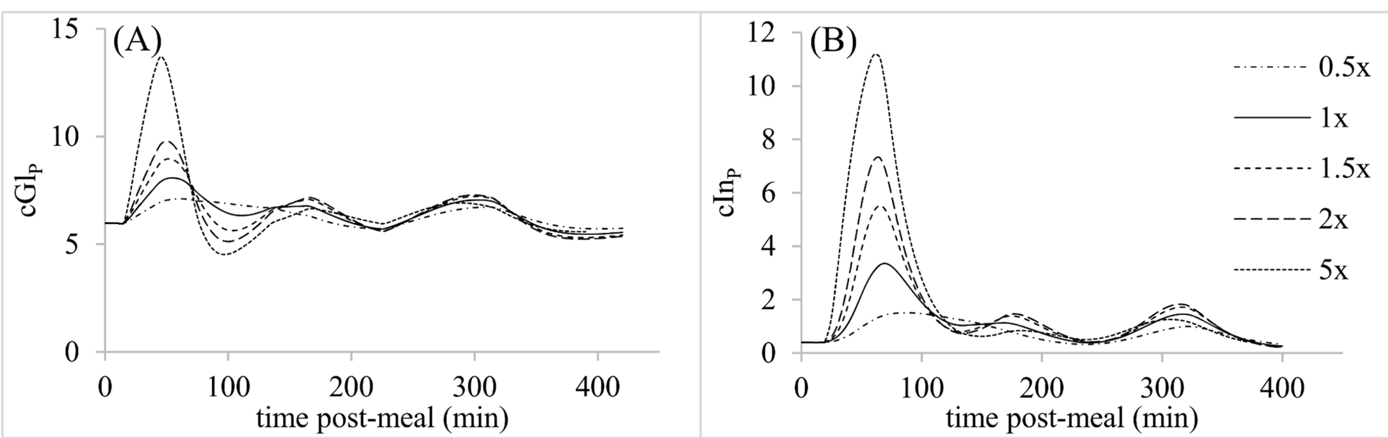

Fig 4. Parameter sensitivity analysis for postprandial first order gastric emptying rate while maintaining a constant ratio of $k_{SP,2}$: $k_{SP,3}$; (A) Predicted plasma glucose concentrations (mM). (B). Predicted plasma insulin concentrations (ng/ml).

doi:10.1371/journal.pone.0156443.g004

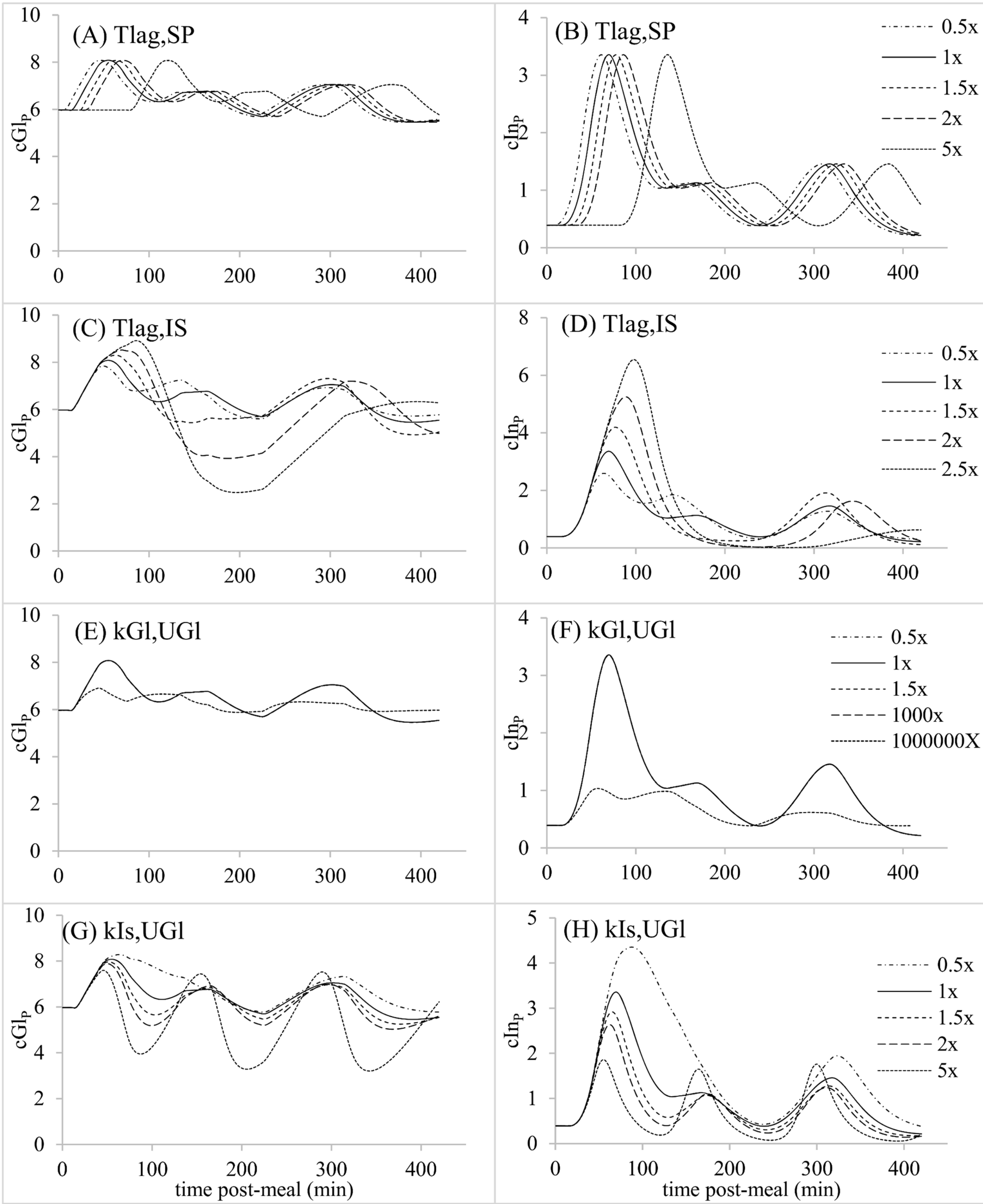

Fig 5. Parameter sensitivity analysis for parameters related to postprandial glucose dynamics; (A,C,E,G) Predicted plasma glucose concentrations (mM). (B,D,F,H). Predicted plasma insulin concentrations (ng/ml).

doi:10.1371/journal.pone.0156443.g005

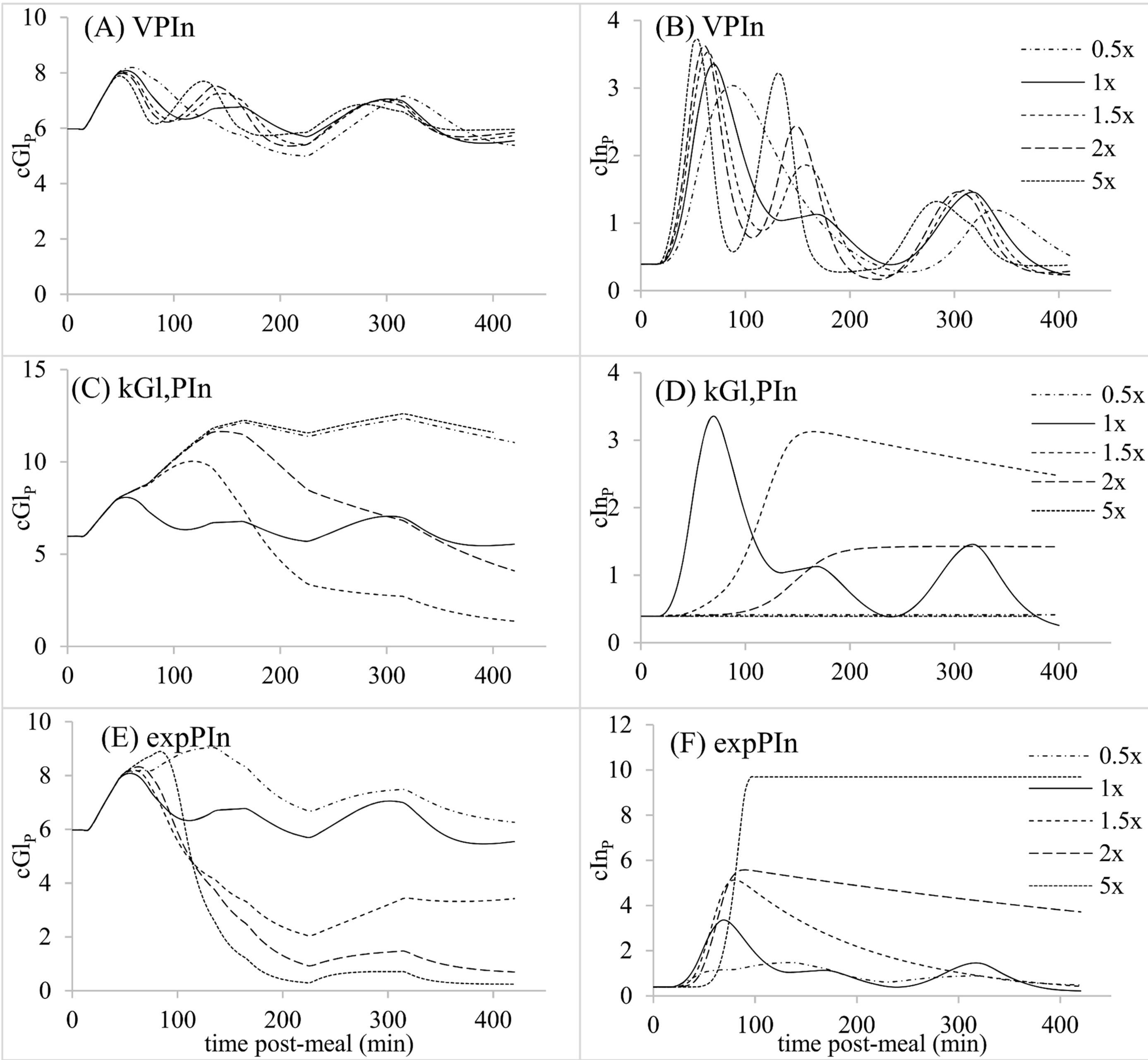

Fig 6. Parameter sensitivity analysis for parameters related to postprandial insulin dynamics; (A,C,E) Predicted plasma glucose concentrations (mM) (B, D,F). Predicted plasma insulin concentrations (ng/ml).

doi:10.1371/journal.pone.0156443.g006

perturbed, the basal steady-state of Gl_P was achieved by setting

$$iPGl_{end} = k_{Gl,UGl} \times {}^{iGl_P}/_{0.251\,BW} + k_{Is,UGl} \times {}^{iIn_P}/_{0.251\,BW} \times {}^{iGl_P}/_{0.251\,BW}, \quad (16)$$

according to eqs 5, 8, 9, 13 and 14.

The predicted reference postprandial patterns of cGl_P and cIn_P each exhibited 3 peaks in association with the gastric emptying profile (Figs 4 to 6). Altering $T_{lag,IS}$, $K_{Gl,PIn}$ and exp_{PIn} changed the number and amplitude of peaks in cGl_P and cIn_P, while k_{SP}, $k_{Gl,UGl}$, $k_{Is,UGl}$ and

V_{PIn} only affected the amplitude of peaks (Figs 4 and 6). Of these latter parameters, $k_{Gl,UGl}$ and V_{PIn} exerted relatively small effects on the amplitude of cGl_P peaks. In addition to affecting wave amplitudes, $T_{lag,IS}$, $k_{Is,UGl}$ and V_{PIn} shifted the times at which cGl_P or cIn_P peaks occurred. $T_{lag,SP}$ also affected peak times without altering other characteristics of the glycemic response (Fig 5). According to these simulations, each of the parameters exerted unique effects on the postprandial responses, except for similarities between $K_{Gl,PIn}$ and exp_{PIn}, and $k_{Is,UGl}$ and V_{PIn}.

Gastric emptying is under neural and hormonal controls that regulate delivery of nutrients to the periphery for metabolism. How much of the glycemic response to a meal is due to gastric emptying rate versus pancreatic responsiveness and tissue insulin sensitivity remains an interesting question for the maintenance of normoglycemia and the attendant therapeutic implications. Because the model contains all three of these control elements, we used it to evaluate the relative role of each in the glycemic response, as indicated by areas under 420-min cGl_P and cIn_P curves (AUC_{Gl} and AUC_{In}, respectively). Results of changing each parameter from 0.5 to 1.5X its reference value are presented as a sensitivity coefficient (SC_A), equal to the fractional change in AUC_A relative to the fractional change in parameter value:

$$SC_A = \left| \frac{AUC_{A(1.5x)} - AUC_{A(0.5x)}}{AUC_{A(1.5x)}} \right| \Big/ \left({}^{(1.5-0.5)}\!/_{1.5} \right). \tag{17}$$

Parameters that affected AUC_{Gl} the most (Table 2) were those related to pancreatic response ($K_{Gl,PIn}$ and exp_{PIn}), followed by insulin sensitivity ($k_{Is,UGl}$, $T_{lag,IS}$) and then gastric emptying (k_{SP}). The sensitivity to $K_{Gl,PIn}$ was 50X that to k_{SP}. However, sensitivity of AUC_{In} was 20X greater than sensitivity of AUC_{Gl} to k_{SP} (Table 2). Pancreatic responsiveness and insulin sensitivity parameters still exhibited stronger effects than the gastric emptying parameter on AUC_{In}, but only by 2X instead of 50X. The difference between insulin and glucose responses to k_{SP} is interesting because one might intuitively presume that both would respond in a similar fashion, given the reciprocal nature of the control paradigm, in which cGl_P affects cIn_P, and cIn_P affects cGl_P. A large effect of k_{SP} on AUC_{In}, with a much smaller effect on AUC_{Gl}, suggests that pancreatic responsiveness is stimulated by faster gastric emptying, so that rapid insulin release (high AUC_{In}) prevents hyperglycemia, leading to low AUC_{Gl}. When gastric emptying was delayed in humans by the amylin analog pramlintide, AUC_{In} over the first 120 min decreased significantly while AUC_{Gl} over the same time period was affected little [20], similar to our simulation results. This decrease was not accompanied by a large change in pancreatic responsiveness parameters [20]. Our simulations show that the pancreatic response to k_{SP} can be independent of changes in the parameters of pancreatic responsiveness V_{In}, $K_{Gl,PIn}$ and exp_{PIn}, because we found an increase in AUC_{In} without altering those parameters. The cause of

Table 2. Reference animal parameter sensitivity coefficients listed from highest to lowest influence on glucose area under the curve.

Parameter	Glucose	Insulin
$K_{Gl,PIN}$	1.643	1.219
exp_{Pin}	1.205	0.819
$k_{Is,UGl}$	0.130	1.338
$T_{lag,IS}$	0.052	0.124
k_{SP}	0.030	0.618
V_{PIn}	0.023	0.075
$T_{lag,SP}$	0.004	0.010
$k_{Gl,UGl}$	0.000	0.000

doi:10.1371/journal.pone.0156443.t002

the higher AUC_{In} as k_{SP} increased in our simulations was the quasi-exponential nature of the effect of cGl_P on insulin release, where small changes in cGl_P exert larger effects on PIn when cGl_P is at high values compared to low.

When k_{SP} was varied in the sensitivity analysis, amplitudes of peaks in cGl_P and cIn_P curves were affected but the number and timing of peaks did not change. Slower gastric emptying might be expected to delay the time to peak cGl_P and cIn_P, as in the pramlintide experiment of Hinshaw et al. [20]. When gastric emptying was set at a continuous rate, by setting all instances of the parameter array Z = 2, then a slower k_{SP} prolonged the time to peak cIn_P (data not shown). Thus, time to peak is a consequence of the rate of gastric emptying (kSP × cGlS), and the number and sequence of fast versus slow or negligible gastric emptying bouts, which we did not present because of the large number of permutations possible.

Parameter estimation

Estimation of $k_{SP,2}$, $k_{SP,3}$ and $k_{Ac,UAc}$ from the 12 cAc_P data points has already been described. The remaining parameters of glucose-insulin dynamics ($k_{Gl,UGl}$, $k_{In,UGl}$, $T_{lag,SP}$ and $T_{lag,IS}$ in eqs 7 to 9, and V_{PIn}, $K_{Gl,PIn}$, exp_{PIn}, and iIn_P in eqs 11, 12 and 14) were estimated with a differential evolution algorithm [21] to minimize the sum of residual sums of squares between predicted and observed cGl_P, and predicted and observed cIn_P. The differential evolution procedure is designed to search for optimal solutions within a large parameter space so that global rather than local minima in RSS are reached. A steady basal state was enforced with eqs 15 and 16. The evolutionary algorithm was run with 80 sets of parameter values in each generation. After 200 generations, means and standard errors of each parameter in the 30 best-fit sets were calculated.

Parameter estimates and their standard errors for each set of animal data are presented in S1 table. According to standard errors, parameter values were significantly different from 0 and were highly identifiable. The fitted aggregate model was able to reproduce postprandial glycemia in the 4 test subjects following the consumption of a milk-based meal with rMSPE percentages ranging from 5 to 15% for glucose (Fig 7) and from 19 to 58% for insulin (Fig 8). Curves with multiple rises and falls in cGlP and cInP were not worse fit than less erratic curves.

Entry rates of glucose into the plasma from intestinal lactose were based on fixed parameters of 100% absorption, 10% conversion of intestinal galactose to GlP, and 90% conversion of intestinal glucose to Gl_P. To test the effect of these parameters on model outputs, we assessed fits to the 4 test subjects with 50% higher iGl_S values than what the fixed parameters predicted. Percentage rMSPE for cGl_P curves were 9.1% on average with the original iGl_S values and 9.5% with the higher iGl_S values, indicating little effect on curve fits.

Discussion

We have presented a novel model for the simulation of postprandial glycemia based on intermittent, exponential gastric emptying with a time lag for appearance of intestinal glucose in plasma, and the minimal models of glucose and insulin dynamics of MINMOD [12]. Dalla Man *et al.* [8] accommodated intermittent gastric emptying in a model of postprandial glycemia as a convolution of three pulses with a second-order decay function for a total of 6 parameters. Other glycemia models with gastric emptying have assumed continuous gastric outflow, where the delay between emptying and appearance in plasma is accommodated with an intermediate intestinal pool from which absorption proceeds according to a first-order rate constant [7,9]. With 2 k_{SP} parameters and a lag-time ($T_{lag,SP}$), our intermittent gastric emptying model of 3 parameters can be considered a minimal model.

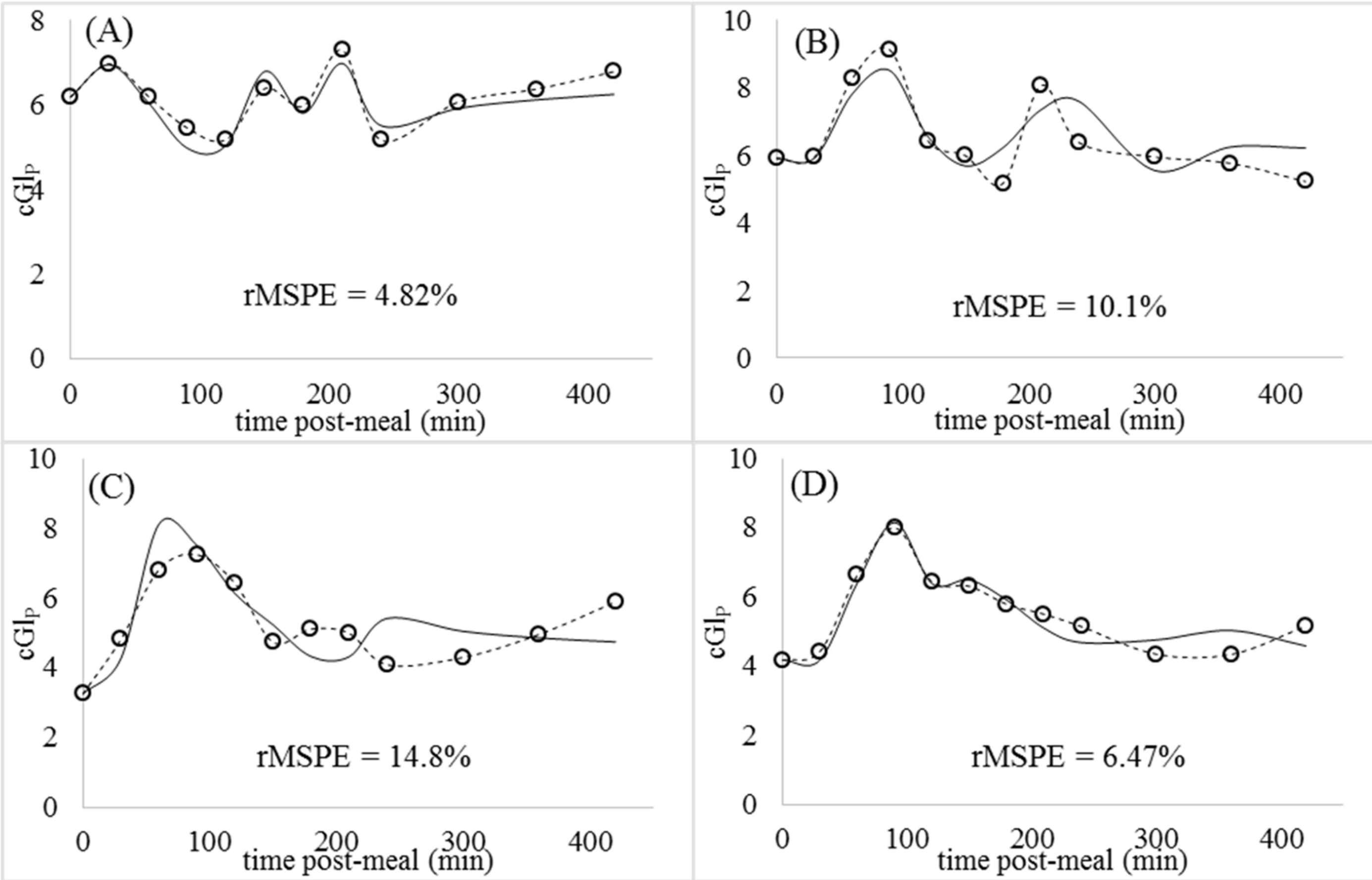

Fig 7. Observed (circles) and predicted (solid lines) postprandial plasma glucose (cGl_P -mM). (A) Animal 1. (B) Animal 2. (C) Animal 3. (D) Animal 4.

doi:10.1371/journal.pone.0156443.g007

We previously developed and assessed mathematical models of gastric emptying using data from horses given various dietary treatments containing Ac [10]. The best fit was achieved by a first-order model of plasma Ac appearance with parameters to describe the duration of periodic gushing of gastric contents as well as the quiescence between gushes. However, the use of two non-zero rate constants to describe gastric emptying rate in the current model provided a simpler solution for improvement of the prediction accuracy of Ac_P appearance. A comparison of Ac and Gl profiles in plasma indicated there was a delay between appearance of the liquid marker in the meal and appearance of glucose. Casein in the meal forms a clot in the stomach that slows its rate of emptying into the small intestine [22,23]. It is possible that the clot retains some of the liquid-associated lactose so that lactose entry into the small intestine is also slowed. However, cumulative bihourly samples of duodenal digesta collected from calves fed clotting or non-clotting milk replacers were not different in lactose content, indicating no effect of the clot on lactose emptying [22]. More frequent samples, as in our datasets [16], may be subject to a clot effect because the reference $T_{lag,SP}$ values we found were less than 30 min. The contribution of lactose hydrolysis to the delay time does not appear to be significant because appearances of ^{13}C label from intact and hydolyzed lactose in a meal consumed by lactose-tolerant humans were identical [24]. This leaves glucose absorption as a potential cause of the delay.

As a percentage of mean observations, prediction errors for cIn_P were higher than for cAc_P or cGl_P. Part of the reason for higher relative errors is the large fold-changes in cIn_P that occur

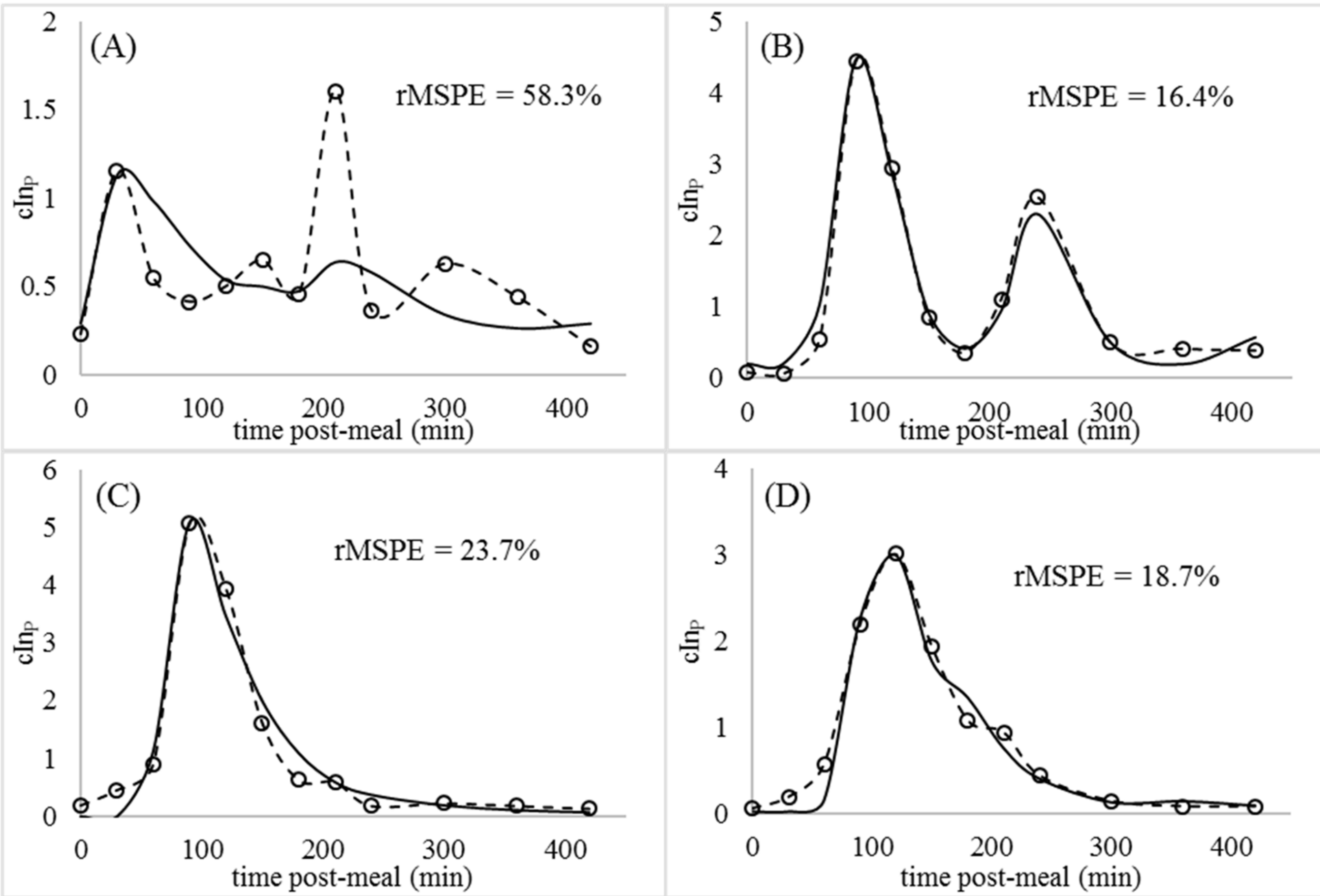

Fig 8. Observed (circles) and predicted (solid lines) postprandial plasma insulin (cIn_P -ng/ml). (A) Animal 1. (B) Animal 2. (C) Animal 3. (D) Animal 4.

doi:10.1371/journal.pone.0156443.g008

during the 420-min timecourse, compared to fold-changes in cAc_P and cGl_P. However, the lower goodness of insulin fits suggests that improvements are possible in the insulin simulations, possibly through consideration of incretin dynamics. Although the model was developed using datasets from calves fed milk, it has utility for predicting post-meal glucose and insulin kinetics in other animal models as well as human subjects.

Conclusion

The combination of minimal models of gastric emptying, plasma glucose dynamics and plasma insulin dynamics suitably describes the erratic postprandial glycemia following a milk-based meal, including depressions below the baseline cGl_P prior to the meal. Sensitivity analysis of the model indicates that faster gastric emptying increases pancreatic responsiveness and keeps plasma glucose concentrations low, independent of the parameters of pancreatic response, simply through the quasi-exponential nature of the relationship between cGl_P and pancreatic insulin release. The model has the potential to be used in the evaluation of dietary treatments for their net effects on pancreatic responsiveness, insulin sensitivity and glucose effectiveness, as well as to predict glycemic responses to various normal-sized meals.

Supporting Information

S1 File. Postprandial plasma acetaminophen (mg/L), glucose (mM) and insulin (ng/ml) of four datasets used for model development.
(XLSX)

S2 File. The acslX program with reference animal parameter values.
(PDF)

S1 Table. Parameters values of four test datasets. Best-fit parameters are shown as mean ± standard error.
(PDF)

Acknowledgments

The authors wish to acknowledge Dr. Larry Schaeffer for assistance in implementing the differential evolution algorithm, and Bill Szkotnicki for his IT expertise.

Author Contributions

Conceived and designed the experiments: PS JPC MAS. Performed the experiments: PS JPC MAS. Analyzed the data: PS JPC. Contributed reagents/materials/analysis tools: JARM MAS. Wrote the paper: PS JPC HB MAS.

References

1. Wolever TMS, Jenkins DJA, Jenkins AL, Josse RG. The glycemic index: methodology and clinical implications. Am J Clin Nutr. 1991; 54:846–854. PMID: 1951155
2. Bergman RN, Ider YZ, Bowden CR, Cobelli C. Quantitative estimation of insulin sensitivity. Am J Physiol. 1979; 236:667–677.
3. Toffolo G, Bergman RN, Finegood DT, Bowden CR, Cobelli C. Quantitative estimation of beta cell sensitivity to glucose in the intact organism. Diabetes. 1980; 29:979–990. PMID: 7002673
4. Janssen P, Vanden Berghe P, Verschueren S, Lehmann A, Depoortere I, Tack J. Review article: the role of gastric motility in the control of food intake. Aliment Pharmacol Ther. 2011; 33:880–894. doi: 10.1111/j.1365-2036.2011.04609.x PMID: 21342212
5. Vist GE, Maughan RJ. The effect of osmolality and carbohydrate content on the rate of gastric emptying of liquids in man. J Physiol 1995; 486:523–531. PMID: 7473216
6. Dedik L, Durisova M, Penesova A, Miklovicova D, Tvrdonova M. Estimation of influence of gastric emptying on shape of glucose concentration-time profile measured in oral glucose tolerance test. Diabetes Res Clin Pr. 2007; 77:377–384.
7. Burattini R, Morettini M. Identification of an integrated mathematical model of standard oral glucose tolerance test for characterization of insulin potentiation in health. Comput Meth Prog Bio. 2012; 107:248–261.
8. Dalla Man C, Caumo A, Cobelli C. The oral glucose minimal model: Estimation of insulin sensitivity from a meal test. IEEE Bio-Med Eng. 2002; 49:419–429.
9. Dalla Man C, Rizza RA, Cobelli C. Meal simulation model of the glucose-insulin system. IEEE Bio-Med Eng. 2007; 54:1740–1749.
10. Cant JP, Walsh VN, Geor RJ. Obtaining information on gastric emptying patterns in horses from appearance of an oral acetaminophen dose in blood plasma. In: Kebreab E., Dijkstra J., Bannink A., Gerrits W. J. J., France J. (eds), Nutrient Digestion and Utilization in Farm Animals: Modelling Approaches, CABI Publishing, Wallingford, UK; 2005. pp. 69–83
11. Clements JA, Heading RC, Nimmo WS, Prescott LF. Kinetics of acetaminophen absorption and gastric emptying in man. Clin Pharmacol Ther 1978; 24:420–431. PMID: 688732
12. Pacini G, Bergman RN. MINMOD: a computer program to calculate insulin sensitivity and pancreatic responsiveness from the frequently sampled intravenous glucose tolerance test. Comput Meth Prog Biomed. 1986; 23:113–122.

13. Bell FR, Razig SAD. Gastric emptying and secretion in the milk-fed calf. J Physiol. 1973; 228:499–512. PMID: 4568910
14. Gannon MC, Khan MA, Nuttall FQ. Glucose appearance rate after the ingestion of galactose. Metabolism. 2001; 50:93–98. PMID: 11172481
15. Barosa C, Silva C, Fagulha A, Barros L, Caldeira MM, Carvalheiro M et al. Sources of hepatic glycogen synthesis following a milk-containing breakfast meal in healthy subjects. Metabolism. 2012; 61:250–254. doi: 10.1016/j.metabol.2011.06.022 PMID: 21862086
16. MacPherson JAR, Berends H, Neal LN, Cant JP, Martin-Tereso J, Steele MA. Effect of plane of nutrition and age on glucose and insulin kinetics and abomasal emptying in female Holstein Friesian dairy calves fed twice daily. J Dairy Sci. In Press
17. Malaisse WJ. Insulin secretion: Multifactorial regulation for a single process of release. Diabetologia.1973; 9:167–173. PMID: 4368828
18. Benshop DL, Cant JP. Developmental changes in clearance of intravenous doses of glucose, acetate and β-hydroxybutyrate from plasma of calves. Livest Sci. 2009; 122:177–185.
19. Xu C, Wensing T, Beynen AC. High intake of calcium formate depresses macronutrient digestibility in veal calves fed milk replacers containing either dairy proteins or whey protein plus soya protein concentrate. J Anim Physiol Anim Nutr. 2000; 83:49–54.
20. Hinshaw L, Schiavon M, Mallad A, Dalla Man C, Basu R, Bharucha AE et al. Effects of delaying gastric emptying on postprandial glucose kinetics, insulin sensitivity and β-cell function. Am J Physiol. 2014; 307:E494–E502.
21. Storn R, Price K. Differential Evolution–A simple and efficient heuristic for global optimization over continuous spaces. J Global Optim. 1997; 11:341–359.
22. Petit HV, Ivan M, Brisson GJ. Duodenal flow of digesta in preruminant calves fed clotting or nonclotting milk replacer. J Dairy Sci. 1987; 70:2570–2576. PMID: 3448108
23. Le Huërou-Luron I, Gestin M, Le Dréan G, Romé V, Bernard C, Chayvialle JA et al. Source of dietary protein influences kinetics of plasma gut regulatory peptide concentration in response to feeding in preruminant calves. Comp Biochem Physiol. 1998; 119A:817–824.
24. Stellaard F, Koetse HA, Elzinga H, Boverhof R, Tjoonk R, Klimp A et al. 13C-Carbohydrate breath tests: impact of physical activity on the rate-limiting step in lactose utilization. Scand J Gastroenterol. 2000; 35:819–823. PMID: 10994620