A New Minimal Mathematical Model of the Endocrine System of Normal Rats Validated against Experimental Data.

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Abstract

As a common laboratory practice, rats are studied as biological models for understanding human physiology. Even though, with the advent of modern computer sciences, new methodologies appeared in medical sciences like simulation and optimization to simplify and improve the experimental work. With this background, a novel simulation model of the endocrine system of Sprague Dawley rats is presented. It is a simplified mathematical model composed of 3 differential equations and 8 parameters that have been determined and validated with experimental measures of plasma glucose and insulin alone by means of calculus and optimization techniques. The results presented here are a step before the development of a type I diabetic model of Sprague Dawley rats.

Keywords: Mathematical modeling, Diabetes Mellitus, In silico rat, Experimental Data

Introduction

Glucose homeostasis is a complex mechanism involving endocrine, autocrine, paracrine and metabolic factors. The result of this homeostatic process is the constancy of the plasma glucose concentration or its variation within very narrow limits, even in states of intake or deprivation of food. This adjusted response is due to the action of hormones such as insulin or glucagon, which could be considered the main effectors of the system. The following diagram (Figure 1) simplifies some of the factors involved in glucose homeostasis.

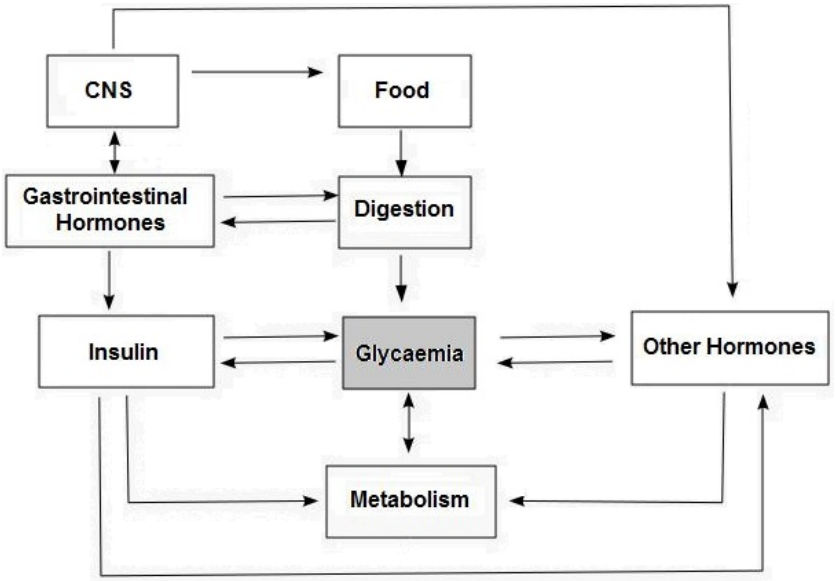

Figure 1: some factors involved in glucose homeostasis.

A detailed study of this issue requires the intervention of mathematical modeling processes that could characterize the system in a quantitative manner and measures of the biological variables involved in the homeostatic system. During the past 40 years it has been developed numerous mathematical models of glucose homeostasis of human beings for different purposes in the field of diabetes mellitus [1,2,3,4], for example: education, to estimate insulin sensitivity, for the development of new drugs, etc. More recently, the race to obtain an artificial pancreas has stimulated the appearance of a kind of models aimed at accelerating the design of glucose control algorithms. One of the main characteristics of this type of simulation models is that they should be able to represent, where possible, the intra and inter-patient variability of the key metabolic parameters in the general population of subjects with type I diabetes mellitus. A possible use of these types of models can be seen in Campetelli et al [5] where a Predictive Functional Control algorithm was applied.

There are many mathematical models that describe the insulin-glucose interaction of the endocrine system. The Sorensen [1] model belongs to the class of complex physiologically based compartment models. The model divides the body into six physiologic compartments: (1) the brain representing the central nervous system, (2) the heart and the lung, which represent the rapidly mixing volumes of the heart, the lung and the arteries, (3) the periphery, which includes the skeletal muscle and adipose tissue, (4) the gut, (5) the liver and (6) the kidneys. Glucose and insulin subsystems are considered separately, with coupling through metabolic effects. This model was originally developed to represent a healthy subject utilizing 22 nonlinear differential equations including 3 equations to describe the endogenous insulin secretion. To simulate a subject with type 1 diabetes, the insulin secretion term is omitted resulting in a model comprising 19 differential equations and 44 parameters. The parameter values were derived from the literature and hence could only represent a nominal ‘average’ virtual subject with type 1 diabetes. As all the parameters of this model are time-invariant the model fails to represent the within subject variability.

Fabietti et al [2] developed a model of the insulin and glucose dynamics in type 1 diabetes to facilitate the design and evaluation of control algorithms for an external artificial pancreas using the subcutaneous route. The model is based on the Bergman’s minimal model. The endogenous insulin secretion is substituted by subcutaneously delivered exogenous insulin and the glucose kinetics is represented by two instead of a single compartment. External inputs of the model such as meals and intravenous glucose boluses have been added together with the submodel of the glucose absorption from the gastrointestinal tract. An interesting feature of the model is the sinusoidal representation of the circadian variability of insulin sensitivity. The amplitude and phase of the circadian rhythm are estimated ‘off-line’ to characterize an individual subject. Another 4 out of 14 model parameters are estimated from clinical data. These include insulin sensitivity, a constant related to the plasma insulin distribution volume and a time constant of the insulin diffusion in the plasma and the remote insulin compartments. Most of the remaining parameters are obtained from the literature or by fitting published data.

The Hovorka [3] model includes two submodels of subcutaneous insulin and subcutaneous glucose kinetics as well as a two-compartment model of the glucose absorption from the gastrointestinal tract. In total, the model is composed of 9 ordinary differential equations and 15 free parameters. An important property of this simulation environment is its ability to represent between and within subject variability. The between subject variability is represented by a population of 18 virtual subjects with type 1 diabetes. The model

parameters were obtained either from clinical studies in subjects with type 1 diabetes or from population probability distributions. The within subject variability of the glucoregulatory system was implemented by superimposing sinusoidal oscillations on a subset of model parameters. A possible weakness of Hovorka virtual patient model is its simple representation of glucose absorption from the gut which may need to be refined.
Dalla Man et al [4] developed a meal-simulation model of glucose–insulin system utilizing data collected in 204 normal subjects who underwent a triple tracer meal protocol. The application of glucose tracers allowed glucose and insulin fluxes during a meal to be calculated. The simulation model is made up of several parsimonious submodels. These unit process models were identified from average data with a forcing function strategy; 35 parameters of the normal subject were estimated. The same strategy was applied to a smaller database containing 14 subjects with type 2 diabetes and the same number of parameters for subjects with type 2 diabetes was estimated. This model is also known as UVa Simulator and has been approved by the FDA in 2008 as a substitute to animal trials in the pre-clinical testing of closed-loop control algorithms [6]. The full model consists of 300 patients with type 1 diabetes. Thanks to models like this one, which contemplate inter-patient variations, a controllability index to preview the possibility of a type 1 diabetic patient to use an artificial pancreas could be done [7].
In diabetic animals the only model available is the Type 1 Diabetes PhysioLab® developed by Entelos in collaboration with the ADA (American Diabetes Association). It is a very detailed and complex computer model (cell level) of the progression of type 1 diabetes in the NOD mouse. The authors have been working with these types of models. In one occasion [8], the Dalla Man model was adjusted to type 1 diabetic rats using experimental data from the laboratory. Another use of models like these could be to test *in silico* fault detection systems applied to erroneous blood glucose measurements using specific biosensors [9].
The main disadvantages of the models mentioned lies in the large number of parameters and the complex metabolic studies that should be performed to know them, which forces to work with average values of many of them, taking away the validity of the results and the applicability of these models.
After facing the difficulties of dealing with large mathematical models with lots of parameters not easily obtainable, the authors of this paper proposes a new model whose strength lies in the possibility of obtaining the value of each parameter for each rat under study, regardless of the use of average values or values obtained with other animals. This model simulates experimental situations very well and has shown that its parameters change in a predictable way from the action of known effectors.

Materials and Methods

The proposed model consists of 3 differential equations that arise from the mass balance of plasma glucose and insulin, considering as disturbing factors the intake of glucose through a diet, the insulin dependent and independent consumption of glucose by the tissues, the hepatic handling and the urinary excretion of glucose. It has only been considered the pancreatic contribution of insulin and its half disappearance by its action on the target tissues.
This rises the following biological model (Figure 2), where the solid lines represent flow of glucose or insulin and the dotted lines the stimulatory (⇢) or inhibitory (⊣) effect.

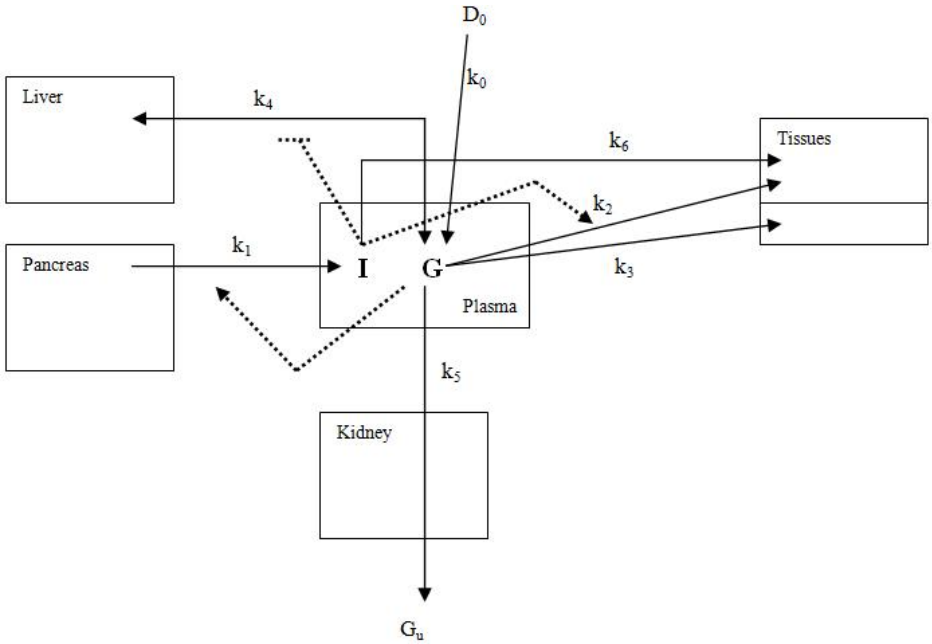

Figure 2: New minimal biological model proposed.

From the biological model proposed the following system of two differential equations can be written:

$$\frac{dI}{dt} = k_1 G - k_6 I \qquad (1)$$

$$\frac{dG}{dt} = -k_4(I - I_{pi}) - k_5(G - G_u) - k_3 - k_2 I + k_0 D \qquad (2)$$

Where G: plasma glucose concentration (mg/dl), I: plasma insulin concentration (pmol/l), D_0: total amount of ingested glucose (mg), D: total amount of glucose in the gastrointestinal tract (mg), k_0: plasma glucose incorporation after an intake constant (dl^{-1} min^{-1}), k_1: pancreatic insulin production rate constant ((pmol/l)/(min.mg/dl)), k_2: insulin dependent glucose utilization by the tissues rate constant (mg/dl)/(min pmo/l), k_3: insulin independent glucose utilization by the tissues rate constant (min^{-1}mg/dl), k_4: uptake (glucogenogenesis) or production of glucose (by glucogenolisis &/or gluconeogénesis) by the liver rate constant (mg/dl)/(min pmo/l), k_5: glucose renal depuration rate constant (min^{-1}), k_6: insulin depuration rate constant (min^{-1}), G_u: glucose threshold, is the plasma glucose concentration at which the renal excretion begins (mg/dl).

The term k_1G represents the pancreatic insulin secretion, which is regulated by the amount of glucose in plasma; k_6I reflects the disappearance of plasma insulin by its action on the target tissues; $k_4(I-I_{pi})$ corresponds to the glucose hepatic handling, being a positive term when insulin level is low ($I<I_{pi}$) indicating the contribution of plasma glucose due to glycogenolysis and gluconeogenesis and negative when it is high ($I>I_{pi}$) indicating the uptake of glucose by the liver for glycogenesis. The term $k_5(G-G_u)$ represents the urinary excretion of glucose. The term k_3 is the insulin independent glucose uptake and k_2I is the insulin dependent glucose uptake by the tissues. Finally, the term k_0D represents the glucose incorporated by the diet.

After a dose (D_0), glucose reaches the gastrointestinal tract (GIT) where the quantity (D) decreases by its absorption and enters plasma (G). In Figure 3 this process is shown were k_e

symbolizes all the processes that consume glucose and k_a the intestinal absorption of glucose.

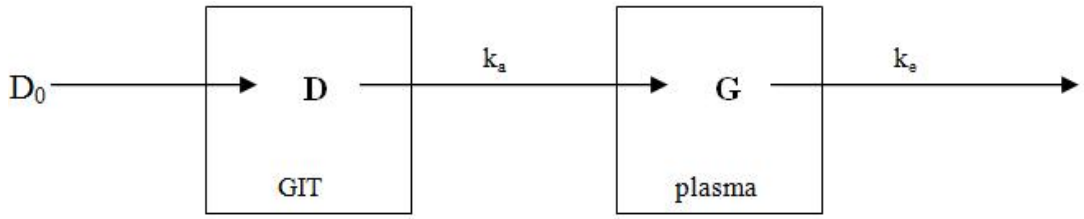

Figure 3: block diagram representation of a meal intake.

D is a function of time and the gastrointestinal absorption (represented by k_a). Assuming that the variation of D as a function of t is of first order, then:

$$\frac{dD}{dt} = -k_a D \Rightarrow D = D_0 e^{-k_a t} \qquad (3)$$

Applying this model to normal individuals the urinary excretion of glucose is zero for which the term represented by k_5 disappears (the blood glucose is below the threshold G_u) resulting in:

$$\frac{dI}{dt} = k_1 G - k_6 I \qquad (4)$$

$$\frac{dG}{dt} = -k_4 (I - I_{pi}) - k_2 I - k_3 + k_0 D \qquad (5)$$

$$\frac{dD}{dt} = -k_a D \qquad (6)$$

Animals

For the laboratory experiments female Sprague Dawley rats were used following the procedures of Rigalli et al [10]. The reason for the use of females lies in the ease of the procedure for obtaining samples of urine by urethral catheterization. The animals were housed in collective cages (4 to 5 animals per cage) with water and balanced food (Gepsa, Argentina) *ad libitum* in the animal facilities of the laboratory. During the experiments, rats were in a temperature-controlled environment at 23-25 ºC, with light-dark cycle 12hr-12hr and filtered airflow at time intervals scheduled. The animals were treated according to accepted international standards for animal handling [11]. In the choosing of models and the number of animals will follow the principle of the 3Rs of Russel and Burch [12].

Oral Glucose Tolerance Test (OGTT)

In animals fasting at least 8 hours blood samples were taken at different times after an oral administration of glucose (0.6 gr. of glucose per 100 gr of body weight) via orogastric tube.

Glucose Measurement

Laboratory determinations of blood glucose were performed using a Perkin Elmer Lambda 11 spectrophotometer with software PECCS on plasma or urine samples using a

commercial kit (Glucose AA, Wiener Laboratories, Rosario) based on the reaction of the enzyme glucose oxidase.

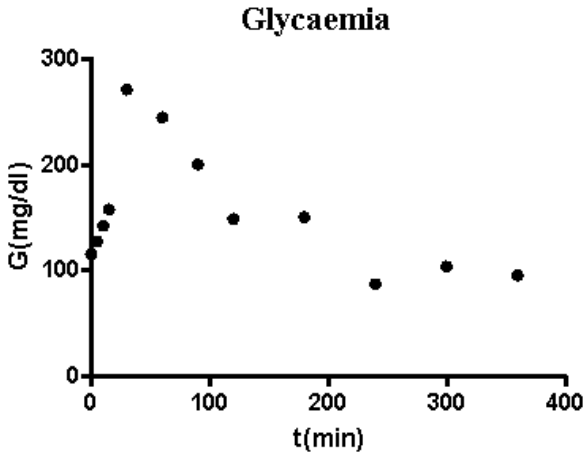

Figure 4: glucose measurements after an OGTT.

Insulin Measurement

The measurement of plasma insulin concentration was performed using a solid scintillation counter Alfanuclear CMOS model using a radioimmunoassay kit specific for rat insulin (Rat insulin Millipore, USA). The test is based on the competition between an insulin sample marked with 125I by specific antibodies against rat insulin. Insulin concentration was measured only in those experiments where the data was strictly necessary. The handling of radioactive material was performed according to the standards established by the Nuclear Regulatory Authority of Argentina (ARN 10.1.1. radiation safety standard).

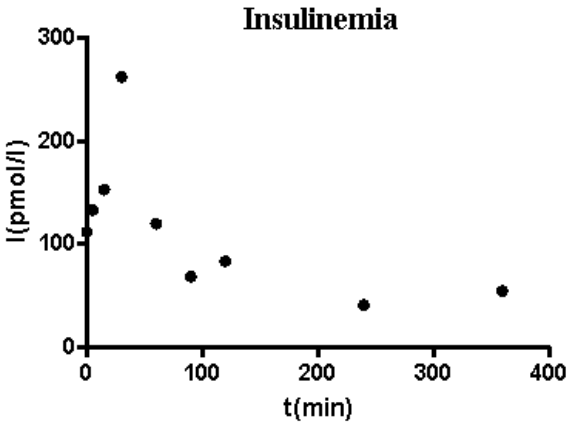

Figure 5: insulin measurements after an OGTT.

Parameter Estimation

For the parameter estimation a series of approximations and assumptions were used to adjust different functions in different regions of the glucose curve vs. time.

Estimation of k_a and k_0

In a fasting animal that eat a given amount of glucose (D_0), at times close to the moment of the intake those processes that consume glucose are negligible with respect to the entry of glucose into plasma. Therefore, the terms that include k_2, k_3 and k_4 can be eliminated. This approach is supported by the significant increase in plasma glucose concentration. According to this simplification, Equation (5) representing the variation of plasma glucose, is reduced to the following:

$$\frac{dG}{dt} = k_0 D \qquad (7)$$

As D is a known function ($D = D_0 e^{-k_a t}$),

$$\frac{dG}{dt} = k_0 D_0 e^{-k_a t} \qquad (8)$$

This is a linear differential equation of first order which could be solved explicitly. So, an equation that represents the plasma glucose concentration versus time, for times close to the administration of glucose is obtained:

$$\int_{Ga}^{G} dG = \int_{0}^{t} k_0 D_0 e^{-k_a t} dt \qquad (9)$$

$$G - G_a = -\frac{k_0 D_0}{k_a}\left(e^{-k_a t} - 1\right) \qquad (10)$$

$$G = G_a + \frac{k_0 D_0}{k_a}\left(1 - e^{-k_a t}\right) \qquad (11)$$

Using this equation to adjust the blood glucose levels measured in the first 3 samples k_0 can be obtained, since k_a is a known value determined by the residuals method [13] and D_0 is a known value. G_a is basal glucose (blood glucose level at time 0).

Estimation of I_{pi}

Once glucose reaches its maximum value (G_{mg}) its decline to reach fasting values again responds to an exponential function of the type:

$$G = G_a + \frac{\left(G_{mg} - G_a\right)}{1 + e^{\left(\frac{\left(t - t_{pi}\right)}{B}\right)}} \qquad \forall t > t_{mg} \qquad (12)$$

where B is a constant whose initial value is adjusted to 10. Here, t_{pi} (the time at which an inflection point is produced in the curve glycaemia vs. Time) could be obtained.

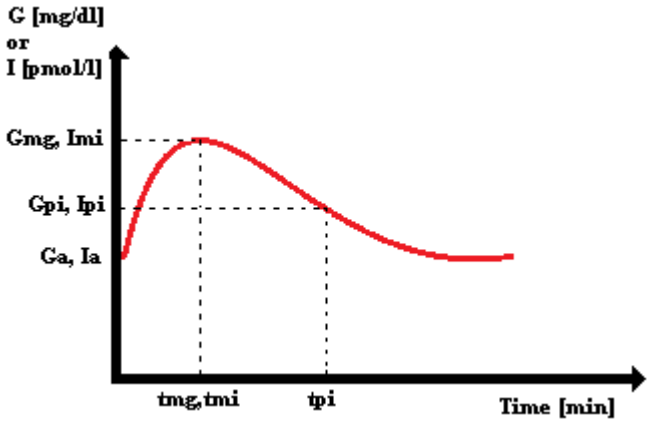

Figure 6: Graphical representation of some values.

The values of G_a and G_{mg} should be fixed. G_a is the basal value, measured at the beginning of the experiment. G_{mg} should be determined by adjusting a second order polynomial near the peak to reduce the error. In Figure 6 the meaning of the values mentioned before is shown.

Once t_{pi} is known, an estimation of I_{pi} could be done. To do so, the data of insulin from its maximum value (I_{mi}) onwards should be adjusted with the following exponential equation:

$$I = (I_{mi} - I_a).e^{-K*(t-t_{mi})} + I_a \qquad (13)$$

Estimation of k_1

Near time zero, the process of insulin depuration with respect to secretion is negligible. So from Equation (4):

$$\frac{dI}{dt} = k_1 G \qquad (14)$$

$$G = G_a + \frac{k_0 D_0}{k_a}\left(1 - e^{-k_a t}\right) \qquad (15)$$

$$\frac{dI}{dt} = k_1\left(G_a + \frac{k_0 D_0}{k_a}\left(1 - e^{-k_a t}\right)\right) \qquad (16)$$

$$\int_0^t dI = \int_0^t k_1\left(G_a + \frac{k_0 D_0}{k_a}\left(1 - e^{-k_a t}\right)\right)dt \qquad (17)$$

$$I - I(0) = \int_0^t k_1 G_a dt + \int_0^t k_1 \frac{k_0 D_0}{k_a} dt - \int_0^t k_1 \frac{k_0 D_0}{k_a} e^{-k_a t} dt \qquad (18)$$

$$I = Ia + k_1 G_a t + k_1 \frac{k_0 D_0}{k_a} t + k_1 \frac{k_0 D_0}{k_a}\left(e^{-k_a t} - 1\right) \qquad (19)$$

Using this function at times close to the glucose intake, k_1 could be obtained.

Estimation of k_2 and k_3

At $t=t_{pi}$, $G=G_{pi}$, $I=I_{pi}$ and $D(t)=0$. So from Equation (5):

$$\frac{dG}{dt}(t_{pi}) = -k_4(I_{pi} - I_{pi}) - k_3 - k_2 I_{pi} \quad (20)$$

$$\frac{dG}{dt}(t_{pi}) = -k_3 - k_2 I_{pi} \quad (21)$$

$$t_{mg} < t \Leftrightarrow G = G_a + \frac{\left(G_{mg} - G_a\right)}{1 + e^{\left(\frac{\left(t - t_{pi}\right)}{B}\right)}} \quad (22)$$

$$\frac{dG}{dt} = \frac{\left(G_a - G_{mg}\right)e^{\frac{(t-t_{pi})}{B}}}{B\left(1 + e^{\left(\frac{t-t_{pi}}{B}\right)}\right)^2} \quad (23)$$

Using the computer software Prisma, with the values of G_a, G_{mg}, B and t_{pi} a curve and a data table could be obtained, which will allow us to compute the derivative of G at two different times close to t_{pi}.

$$\frac{dG}{dt}(t_1) = -k_3 - k_2 I_1 \quad (24)$$

$$\frac{dG}{dt}(t_2) = -k_3 - k_2 I_2 \quad (25)$$

$$k_2 = \frac{\frac{dG}{dt}(t_1) - \frac{dG}{dt}(t_2)}{I_2 - I_1} \quad (26)$$

$$k_3 = -\frac{dG}{dt}(t_1) - k_2 I_1 \quad (27)$$

Estimation of k_4

Prolonged fasting blood glucose is constant so dG/dt=0, D=0, the insulin dependent glucose utilization ($k_2I(t)$) is nearly 0, and therefore from Equation (5):

$$\frac{dG}{dt} = -k_4(I_a - I_{pi}) - k_3 = 0 \quad (28)$$

$$k_3 = -k_4(I_a - I_{pi}) \quad (29)$$

$$k_4 = \frac{k_3}{I_{pi} - I_a} \quad (30)$$

Estimation of k_6

Finally, when insulinemia is at its maximum (I_{mi}), $G=G_{mi}$ and dI/dt=0, so from Equation (4) we have:

$$k_1 G_{mi} - k_6 I_{mi} = 0 \quad (31)$$

$$k_6 = \frac{k_1 G_{mi}}{I_{mi}} \quad (32)$$

Results

Even though this methodology has been validated with many rats, in this work to determine the model parameters the methodology will be applied to one Sprague Dawley rat. After an oral glucose tolerance test with an intake of 1400 mg of glucose, plasma glucose and insulin measurements are shown in Table 1:

t [min]	G[mg/dl]	I[pmol/l]
0.00	115.20	111.03
5.00	126.80	132.30
10.00	141.80	71.33
15.00	157.30	152.01
30.00	271.00	261.79
60.00	244.60	119.20
90.00	200.00	67.91
120.00	148.20	82.66
180.00	149.80	156.65
240.00	86.30	40.08
300.00	103.20	117.00
360.00	94.90	53.69

Table 1: experimental measurements of glucose and insulin.

With this data, the values explained in Figure 6 were calculated and their results are shown in Table 2:

$\mathbf{G_{mg}}$	254.2507
$\mathbf{t_{mg}}$	62.02247
$\mathbf{t_{pi}}$	99.98
$\mathbf{I_{mi}}$	190.4072
$\mathbf{t_{mi}}$	41.53846
$\mathbf{G_{mi}}$	91.0389599

Table 2: values used to estimate some model parameters.

Using this data, parameters were calculated as shown in the previous section. With these estimations as initial values, an optimization algorithm was finally applied to refine the fitting of the data. In Table 3 the values of the parameters with these approaches can be seen. To perform the optimization, the Matlab toolbox Diffpar was used. In Figure 7 the result of the optimization is shown.

Parameters	Estimated	Optimized
k1	0.1774	0.1774
k2	2.36E-02	1.89E-04
k3	3.4228	0.4783
k4	0.0643	0.03432
ka	0.0265	0.0265
k6	0.2303	0.2606
k0	0.00012	0.0072
Ipi(pmol/l)	68.82	
Ia(pmol/l)	111.03	
Ga(mg/dl)	115.2	
Do(mg)	1400	

Table 3: Model parameters.

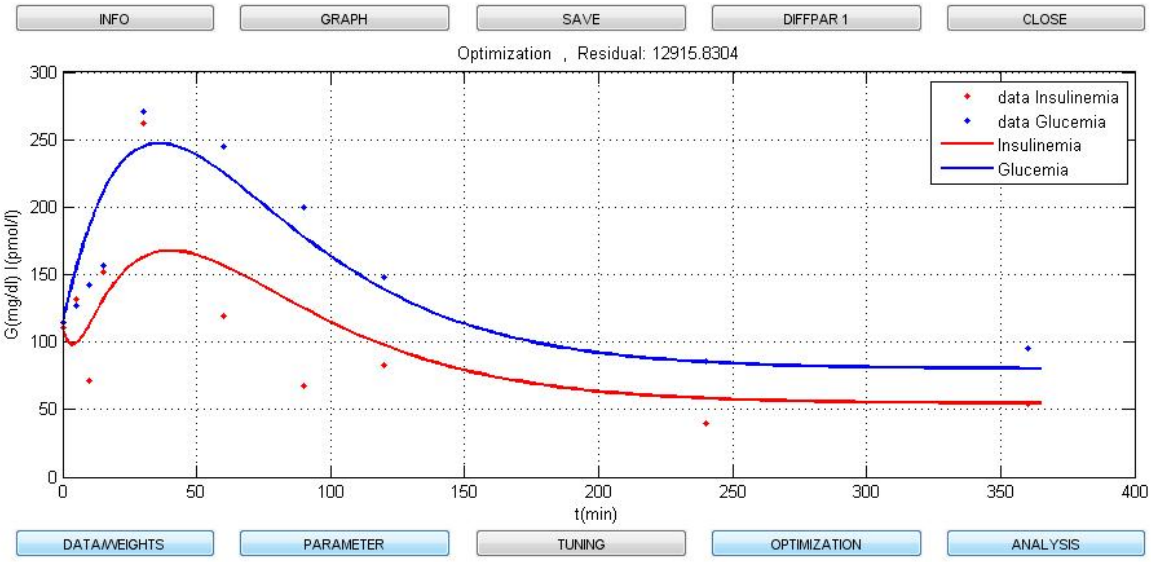

Figure 7: Model parameters fitting using Matlab.

Discussion

In view of the difficulties of obtaining detailed mathematical physiological models of the endocrine system, which requires measurements of tracers and drugs that need specific and expensive equipments, the availability of simpler models easily obtainable in any laboratory is of prime necessity, mainly for developing countries. In this paper a unique mathematical model of the endocrine system of normal rats has been presented. It is based on experimental data and showed good agreement when simulating with computers. To make a comparison with the complex mathematical model used before by the authors [8], it is worth mentioning that the number of parameters in that case was 30 in contrast to 7 in this new model. In future, the modifications to contemplate the responses of diabetic rats will be studied. It will be very useful to help scientists accelerate the development of an artificial pancreas for everyday use in humans.

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