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Mathematical model of glucose–insulin homeostasis in healthy rats

Mercedes Lombarte [a,*], Maela Lupo [a], German Campetelli [b], Marta Basualdo [b], Alfredo Rigalli [a]

[a] Bone Biology Laboratory-School of Medicine, Rosario National University, Santa Fe 3100, Rosario, Santa Fe, Argentina
[b] GIAIP-CIFASIS-CONICET-Rosario National University, 27 de febrero 210 bis, Rosario, Santa Fe, Argentina

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ABSTRACT

According to the World Health Organization there are over 220 million people in the world with diabetes and 3.4 million people died in 2004 as a consequence of this pathology. Development of an artificial pancreas would allow to restore control of blood glucose by coupling an infusion pump to a continuous glucose sensor in the blood. The design of such a device requires the development and application of mathematical models which represent the gluco-regulatory system. Models developed by other research groups describe very well the gluco-regulatory system but have a large number of mathematical equations and require complex methodologies for the estimation of its parameters. In this work we propose a mathematical model to study the homeostasis of glucose and insulin in healthy rats. The proposed model consists of three differential equations and 8 parameters that describe the variation of: blood glucose concentration, blood insulin concentration and amount of glucose in the intestine. All parameters were obtained by setting functions to the values of glucose and insulin in blood obtained after oral glucose administration. *In vivo* and *in silico* validations were performed. Additionally, a qualitative analysis has been done to verify the aforementioned model. We have shown that this model has a single, biologically consistent equilibrium point. This model is a first step in the development of a mathematical model for the type I diabetic rat.

1. Introduction

Glucose homeostasis is a complex mechanism involving endocrine, autocrine, paracrine and metabolic factors. This homeostatic process results in constancy of blood glucose concentration or in its variation within very narrow limits, even in intake or deprivation of food states. This adjusted response is due to the action of hormones such as insulin, which could be considered the main effectors of the system.

Deficiency in insulin production or the lack of tissue response to it generates well-characterized clinical conditions such as DMTI and DMTII. DMTI treatment usually requires the administration of insulin; this may be done with single injections or using infusion pumps. These devices are programmed according to the activities and meals of the patient, obtaining good results. The final solution for the treatment of diabetes would be an infusion pump coupled to a continuous glucose sensor in blood, constituting an artificial pancreas. The development of this technology requires the intervention of mathematical modeling processes that characterize the system in a quantitative way, and measurement of biological variables involved in the homeostatic system.

During the past 40 years numerous mathematical models of the gluco-regulatory system have been developed in the field of diabetes with different purposes. These mathematical models have been geared towards the design of controllers of glucose for obtaining an artificial pancreas. One of the main characteristics of this type of simulating models is that they should be able to represent, where possible, the intra and inter patient variability of their parameters.

There are many mathematical models that describe the insulin–glucose interaction of the endocrine system. The Sorensen [1] model divides the body into compartments. This model was originally developed to represent a healthy subject utilizing 22 nonlinear differential equations including 3 equations to describe the endogenous insulin secretion. The parameter values were derived from the literature and hence could only represent a nominal 'average' virtual subject with DMTI. As all the parameters of this model are time-invariant the model fails to represent the within-subject variability.

The Fabietti [2] model is a dynamic model of glucose–insulin specifically conceived to facilitate the design and evaluation of control algorithms. It is based in the minimal model of Bergman [3]. In this model endogenous insulin secretion is substituted by insulin subcutaneous injection, and glucose kinetics is represented

Abbreviations: DMTI, diabetes mellitus type I; DMTII, diabetes mellitus type II; NaF, sodium fluoride; SE, standard error.

* Corresponding author. Tel.: +54 341 4804558/63.
E-mail address: mercedes_lombarte@yahoo.com.ar (M. Lombarte).

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by two compartments. External inputs of the model such as meals and glucose administration have been added in the sub-model of glucose absorption from the gastrointestinal tract. An interesting feature of the intestinal absorption model is that it allows distinguishing between fast and slow absorption carbohydrates. Another interesting feature is the sinusoidal representation of the circadian variability of insulin sensitivity. Four of 14 parameters of the model were estimated from clinical data. The others parameters of the model were obtained from fitting data published in the literature.

The Hovorka [4] model includes three compartment sub-models: subcutaneous insulin kinetics, subcutaneous glucose kinetics, and glucose absorption from the gastrointestinal tract. On the whole, the model is composed of 9 ordinary differential equations and 15 parameters. The parameters of the model were obtained either from clinical studies in subjects with type 1 diabetes or from population probability distributions.

The UVa Simulator [5] was developed from data collected from 204 normal subjects who participated in a protocol with isotopically labeled glucose. The use of such tracers allowed the measurement of the flows of glucose and insulin after ingestion of a mixed meal. Subsequently, the model was adapted to the diabetic population with data obtained from the literature. On the whole, the model has 13 ordinary differential equations and 26 parameters. The greatest novelty of this model is a more detailed description of the transit of glucose through the intestine.

The main disadvantages of the mentioned models lie in the large number of parameters and the complex metabolic studies that should be performed to estimate them. This compels to work with average values that decrease the validity of the results and the applicability of the models.

In this work we propose a mathematical model to study the homeostasis of glucose and insulin in healthy rats. Additionally, a quantitative and qualitative analysis has been done to verify the aforementioned model. The values of the rate constants for the homeostatic processes were obtained using experimental data of blood glucose and insulin. Finally, the validation of the model was achieved using situations with known effects over glucose and insulin homeostasis. For example: NaF and physical activity. The NaF is an important disrupting component of the system that produces inhibition of insulin secretion after a dose [6,7]. Physical activity causes increased response of insulin receptors, improving the effect of this hormone in target tissues. Physical activity rises glucose uptake by muscle tissue by increasing the number of GLUT4 transporters [8], producing a higher insulin sensitivity [9].

The advantage of this model lies in the possibility of obtaining all the parameters for each animal. In addition, the parameters are obtained from blood glucose and insulin levels, common biochemical measurements.

2. Model formulation

Fig. 1 shows a representative diagram of the biological model used for the development of the mathematical model.

The proposed model consists of three differential equations that describe the variation of: blood glucose concentration (G), blood insulin concentration (I) and amount of glucose in the intestine (D).

$$dI/dt = k_1 G - k_6 I \tag{1}$$

$$dG/dt = -k_4(I - I_{pi}) - k_2 I - k_3 + k_0 D \tag{2}$$

$$dD/dt = -k_a D \tag{3}$$

System 1

Equation (1) represents the variation of blood insulin concentration. The term $k_1 G$ represents the pancreatic insulin secretion, which is regulated by blood glucose concentration; and the $k_6 I$ term represents blood insulin clearance.

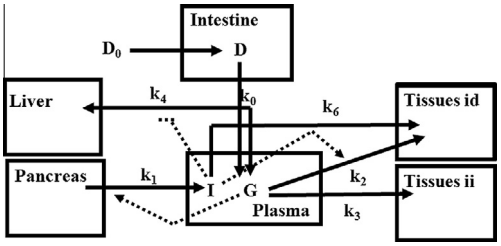

Fig. 1. The glucose–insulin system. The solid lines represent flow of glucose or insulin and the dotted lines the stimulatory (·····▶) or inhibitory (·····┤) effect. G: blood glucose concentration (mg dl^{-1}), I: blood insulin concentration (pmol l^{-1}), D_0: glucose intake (mg), k_0: rate constant of blood glucose incorporation from diet (dl^{-1} min^{-1}), k_1: rate constant of insulin secretion (pmol dl min^{-1} mg^{-1} l^{-1}), k_2: rate constant of insulin-dependent glucose uptake by the tissues (mg l dl^{-1} min^{-1} pmol^{-1}), k_3: rate constant of insulin-independent glucose uptake by the tissues (mg min^{-1} dl^{-1}), k_4: rate constant of uptake (glycogenesis) or production of glucose (by glycogenolysis and/or gluconeogenesis) by the liver (mg l dl^{-1} min^{-1} pmol^{-1}), k_6: rate constant of blood insulin clearance (min^{-1}).

Equation (2) represents the variation of blood glucose concentration. The term $k_4(I - I_{pi})$ models the hepatic handling of glucose. It is a positive term when the blood insulin concentration is lower than I_{pi} (indicating the contribution of blood glucose by glycogenolysis and gluconeogenesis) and a negative term when blood insulin concentration is higher than I_{pi} (indicating the uptake of glucose by the liver for glycogenesis, glycolysis, or synthesis of lipids). I_{pi} is a parameter that represents the blood insulin concentration when the liver changes from the uptake to the production of glucose. The k_3 term represents the insulin-independent glucose uptake by the tissues. The $k_2 I$ term represents the insulin-dependent glucose uptake by the tissues and $k_0 D$ the variation in blood glucose concentration due to the oral administration of glucose.

Equation (3) represents the variation of glucose in the intestine, where the k_a parameter is the rate constant of glucose absorption.

3. Mathematical analysis

We performed the study of the characteristics of the solutions by analyzing the nature of the eigenvalues and eigenvectors of the matrix of the coefficients.

The proposed system is:

$$\begin{cases} dI/dt = -k_6 I + k_1 G \\ dG/dt = -(k_2 + k_4)I + k_0 D - k_3 + k_4 I_{p_i} \\ dD/dt = -k_a D \end{cases}$$

System 2

Subject to the following initial conditions

$$I(0) = I_a; \quad G(0) = G_a; \quad D(0) = D_0$$

The differential equations system presented is a linear system of the first order, inhomogeneous with constant coefficients; ensuring the existence and uniqueness of a solution to the initial values problem posed.

The stationary or equilibrium points of System 2 are obtained by solving the following algebraic system:

$$-k_6 I + k_1 G = 0 \tag{4}$$

$$-(k_2 + k_4)I + k_0 D - k_3 + k_4 I_{p_i} = 0 \tag{5}$$

$$-k_a D = 0 \tag{6}$$

System 3

The following values were obtained.

From Equation (6) $D^* = 0$

Replacing in Equation (5) was obtained:

$$I^* = \frac{k_4 I_{p_i} - k_3}{k_2 + k_4}$$

Replacing in Equation (4) was obtained:

$$G^* = k_6 I^* / k_1$$

Thus the equilibrium point is: $(I^*;G^*;D^*)$

Furthermore, if we consider that: $D^* = 0$, G^* is fasting blood glucose concentration and I^* is fasting blood insulin concentration.

From this analysis and considering that $I > 0$ we reached the following condition:

$$k_4 I_{p_i} - k_3 > 0$$

This condition was verified in each animal when the model was tested *in vivo* (see below).

The matrix of coefficients of System 2 is:

$$A = \begin{pmatrix} -k_6 & k_1 & 0 \\ -(k_2 + k_4) & 0 & k_0 \\ 0 & 0 & -k_a \end{pmatrix}$$

The eigenvalues of this matrix are:

$$\lambda_1 = -k_a$$
$$\lambda_2 = -k_6/2 + \sqrt{\Delta}/2 \quad \text{where } \Delta = k_6^2 - 4k_1(k_2 + k_4)$$
$$\lambda_3 = -k_6/2 - \sqrt{\Delta}/2$$

The nature of the equilibrium depends on the characteristics of these eigenvalues. There are three possibilities:

Case 1: If $\Delta > 0$, the three eigenvalues will be real numbers, different and negatives; hence the stationary point is an asymptotically stable node.

Case 2: If $\Delta < 0$ the two eigenvalues will be complex numbers with a negative real part; hence the stationary point is a stable spiral point.

Case 3: If $\Delta = 0$ two eigenvalues will be real, negatives and equal. The equilibrium point is an attractor. The character of the critical point depends on the existence or not of two linearly independent eigenvectors.

Plots of blood glucose concentration and blood insulin concentration were obtained corresponding to case 1 and 2 (Fig. 2) in the *in vivo* experiments. Case 3 was not observed.

4. Parameter estimation

The parameters were obtained by setting functions to the values of glucose and insulin in blood obtained after oral glucose administration.

4.1. Estimation of k_a and k_0

Immediately after the ingestion of a dose of glucose (D_0) the processes that consume glucose are negligible in respect to the entry of glucose into blood. Therefore, Equation (2) of the System 1 is reduced to the following:

$$dG/dt = k_0 D \tag{7}$$

The glucose amount in the digestive system is represented by Equation (8). Equation (9) is obtained by replacing Equation (8) in Equation (7):

$$D = D_0 e^{-k_a t} \tag{8}$$

$$dG/dt = k_0 D_0 e^{-k_a t} \tag{9}$$

The solution of Equation (9) represents blood glucose concentration as a function of time, for times close to the administration of glucose (Equation (10)):

$$G = G_a + \frac{k_0 D_0}{k_a}(1 - e^{-k_a t}) \tag{10}$$

Using this equation to fit the values of blood glucose concentration between 0 and 15 min k_0, is obtained. The parameter k_a is obtained by the method of residuals [10]; D_0 is the amount of glucose orally administered, and G_a is fasting blood glucose level (blood glucose level at time 0).

4.2. Estimation of I_{pi}

Once glucose reaches its maximum blood value (G_{Mg}), it declines to reach fasting values again and responds to a sigmoid function as described by Equation (11):

$$G = G_a + \frac{(G_{Mg} - G_a)}{1 + e^{\left(\frac{(t - t_{pi})}{B}\right)}} \tag{11}$$

B is a constant characteristic of each animal. Employing the Equation (11) to fit blood glucose data from its maximum value forward, the value of t_{pi} is obtained. This value is the time at the inflection point in blood glucose concentration as a function of time. The value of blood insulin levels at this time is an estimate of the parameter named I_{pi}.

G_{Mg} is the maximum concentration of glucose obtained after an oral intake of glucose; this value is obtained by fitting a polynomial function of degree 2. This nonlinear fitting is done for blood glucose levels close to maximum blood concentration.

With the value of t_{pi}, the I_{pi} can be estimated. This data of insulinemia should be adjusted with the exponential equation (Equation (12)) from its maximum value (I_{Mi}) onwards.

$$I = (I_{Mi} - I_a).e^{-K^*(t - t_{Mi})} + I_a \tag{12}$$

4.3. Estimation of k_2 and k_3

At t_{pi} time $G = G_{pi}$, $I = I_{pi}$ and $D = 0$. Then Equation (2) can be written:

$$dG/dt(t_{pi}) = -k_4(I_{pi} - I_{pi}) - k_3 - k_2 I_{pi}$$

$$dG/dt(t_{pi}) = -k_3 - k_2 I_{pi} \tag{13}$$

Changes in blood glucose levels close to t_{pi}, can be obtained calculating the derivative from Equation (11).

$$dG/dt = \frac{(G_a - G_{mg}) e^{\frac{(t - t_{pi})}{B}}}{B\left(1 + e^{\left(\frac{t - t_{pi}}{B}\right)}\right)^2} \tag{14}$$

Equation (14) allows to calculate dG/dt at two different times (t_1 and t_2) near to t_{pi}; with the values of blood insulin levels for these times (I_1 and I_2), which are calculated using Equation (12), a linear equation system was obtained (System 4). The k_2 and k_3 parameters can be obtained by solving this system.

$$\begin{cases} dG/dt(t_1) = -k_3 - k_2 I_1 \\ dG/dt(t_2) = -k_3 - k_2 I_2 \end{cases}$$

System 4

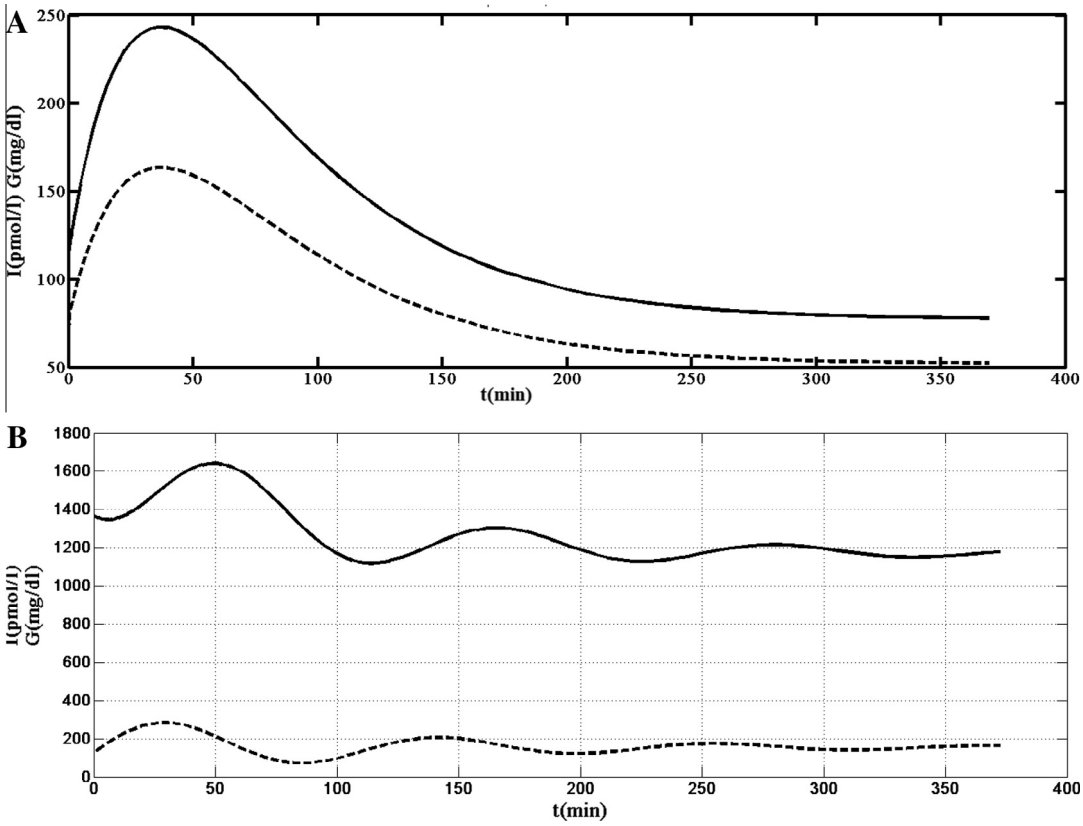

Fig. 2. Blood glucose concentration and blood insulin concentration as a function of time. (A) simulation obtained from parameters of a rat that represents case 1. (B) simulation obtained from parameters of a rat that represents case 2. The insulin curves are shown in solid line and glucose curves are represented by dotted line in both Figures A and B.

4.4. Estimation of k_4

In conditions of fast, $D = 0$ and blood glucose level is constant; as a consequence $dG/dt = 0$, insulin-dependent glucose uptake ($k_2 I$) could be considered negligible. Therefore, from Equation (2) of System 1, Equation (15) was obtained:

$$k_3 = -k_4(I_a - I_{pi}) \tag{15}$$

The parameter k_4 can be estimated, since I_a, I_{pi} and k_3 are known.

4.5. Estimation of k_1

Immediately after glucose administration, the process of blood insulin depuration in respect to secretion is negligible. So Equation (1) of System 1 is modified:

$$dI/dt = k_1 G \tag{16}$$

Blood glucose at times close to the intake of glucose is represented by Equation (10). Combining Equation (16) and Equation (10) and solving, Equation (17) was obtained.

$$I = Ia + k_1 G_a t + k_1 \frac{k_0 D_0}{k_a} t + k_1 \frac{k_0 D_0}{k_a^2}\left(e^{-k_a t} - 1\right) \tag{17}$$

I_a is the fasting blood level of insulin and the other parameters have been previously defined.k_1 is obtained by fitting Equation (17) to the values of insulinemia at times close to the time of the administration of glucose.

4.6. Estimation of k_6

Finally, when insulinemia is at its maximum (I_{Mi}), $G = G_{Mi}$ y $dI/dt = 0$, so, from Equation (1) of System 1 we have:

$$k_1 G_{Mi} - k_6 I_{Mi} = 0$$

In this equation, k_1, I_{Mi} y G_{Mi} (G_{Mi}: blood glucose at the time at which insulin is maximum) are known values, so we can obtain the k_6 parameter. I_{Mi} is obtained with the same method used to obtain G_{Mg} (a fit with a second-degree polynomial function). G_{Mi} can be calculated with Equation (11) at the time of the maximum insulin value.

4.7. Parameter optimization

Optimization was done with the Edsberg and Wedin DIFFPAR (DIFFerential equations with unknown PARameters) pack. The toolbox DIFFPAR is based on formulating the parameter estimation problem as a non-linear weighted least squares problem. A Gauss–Newton type algorithm with local regularization is used for minimizing an objective function [11]. Optimization was performed taking the estimated values as initial values in 7 healthy rats. Fig. 3 displays the values of estimated parameters with the described methodology. The dispersion of the results supports the hypothesis that the values of parameters must be calculated for each animal.

5. Numerical simulation

In order to know the system behavior, a simulation was done using the MatLab Simulink library. Simulink has a graphical interface that allows building models in a block diagram, and includes

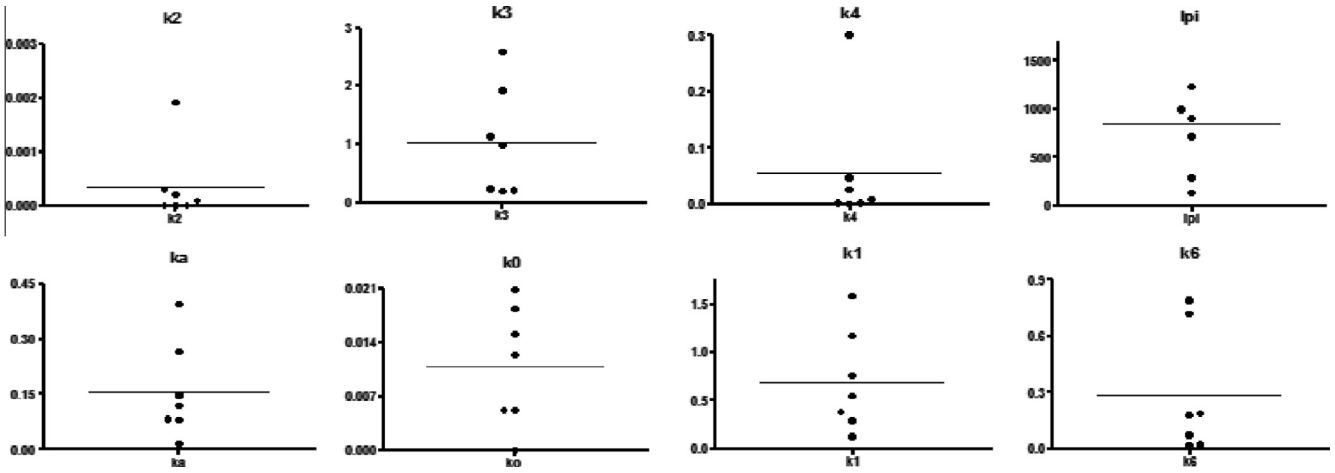

Fig. 3. Optimized values of parameters. The points show the values of the parameters obtained with the described methodology ($n = 7$). The horizontal line in each plot represents the average for each parameter.

an extensive library of input and output blocks, mathematical operations, linear components and connectors. The use of display blocks (Scopes) allows us to observe graphically the results of the simulation. It is also possible to change the value of the parameters and to control changes in exploration. Simulation plots were made with the values of the parameters estimated in rats and then theoretical modifications were generated thereof. In this paper we show data from simulation with two glucose intakes (1500 and 2500 mg), with two values of k_1 (parameter representing pancreatic secretion) and k_2 (parameter describing the insulin-dependent glucose uptake by the tissues).

Fig. 4 shows the plots of blood glucose and blood insulin that were obtained from simulation with optimized parameters in healthy rats, after the intake of two different amounts of glucose. As expected, higher intakes of glucose produce higher blood levels of glucose and insulin.

Fig. 5 shows the plots of simulation that were obtained with different values of parameter k_1.

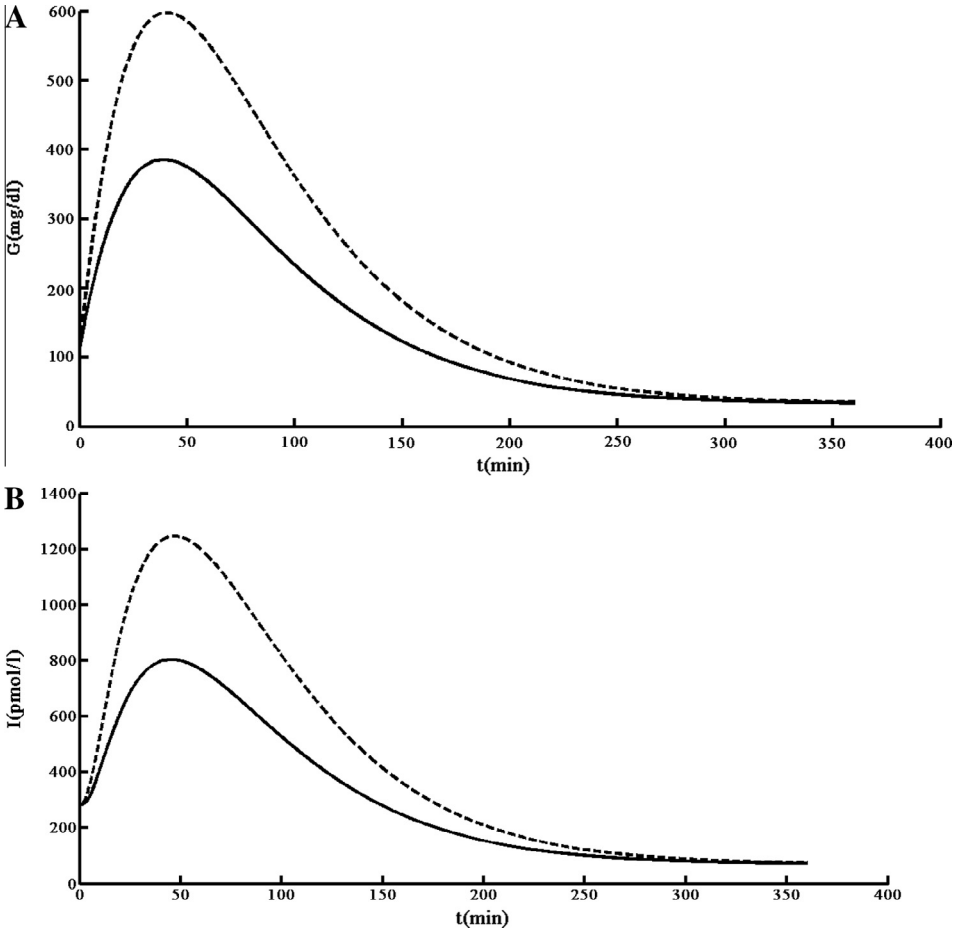

Fig. 4. Simulation with different intake of glucose. The graph (A) shows the simulation of blood glucose levels as a function of time and the graph (B) shows blood insulin levels as a function of time. The full line shows the behavior after a dose of 1500 mg glucose and the dotted line when the dose of glucose is increased to 2500 mg.

The decrease in value of parameter k_1 represents a decrease of insulin secretion. This state could be observed in the early stage of type 1 diabetic rats, when plasma glucose level is not higher than renal glucose threshold. As seen in the graphs, the behavior of the proposed model reflects the expected one. When simulating, a lower value of k_1 produces lower levels of insulin and higher levels of glucose in blood.

Fig. 6 shows the simulation obtained when parameter k_2 increases. This parameter represents the rate constant of insulin-dependent glucose uptake by the tissues. As expected, the simulation with higher values of k_2 reflected lower values of glucose and insulin. This situation was also used as validation *in vivo*, increasing the value of k_2 by subjecting rats to physical activity.

6. Validation *in vivo*

In vivo experiments were carried out to validate the values of k_1, k_2 and k_6 parameters.

6.1. Validation of parameter k_1

The parameter k_1 is the rate constant of insulin secretion. The parameter was validated throughout the administration of NaF, because it has been previously demonstrated that this substance reduces insulin secretion [6]. Parameters were estimated and optimized, using the methodology described, in two experimental groups: Controls ($n = 7$) and NaF ($n = 4$, orally treated with 40 μmol/100 g of NaF before glucose administration). The parameters were obtained with the same methodology in both experimental groups, and then the values of the parameter k_1 were compared with Student's t test.

Fig. 7 shows a significant decrease in parameter k_1 in animals that were treated with NaF, $p = 0.0251$.

6.2. Validation of parameter k_2

The parameter k_2 is the rate constant of insulin-dependent glucose uptake by the tissues. It is known that exercise increases insulin receptors and sensitivity to the hormone [8,9]. The parameter was estimated and optimized in a sedentary group of 7 rats (Controls) and in 4 rats (Exercise group) that did exercise in a treadmill, 30 min/day, 2, 5 m/seg, for 30 days. The values of k_2 were compared with Student's t test.

The Fig. 8 shows a significant increase in parameter k_2 in animals that perform physical activity for 30 days, $p = 0.0071$.

Simulations were performed with the values of k_1 and k_2 obtained before, for control and treated groups. Fig. 9 shows the insulin and glucose behavior when the parameters k_1 and k_2 are changed.

6.3. Validation of parameter k_6

The parameter k_6 is the rate constant of blood insulin clearance. The validation of parameter k_6 was done by the estimation of this

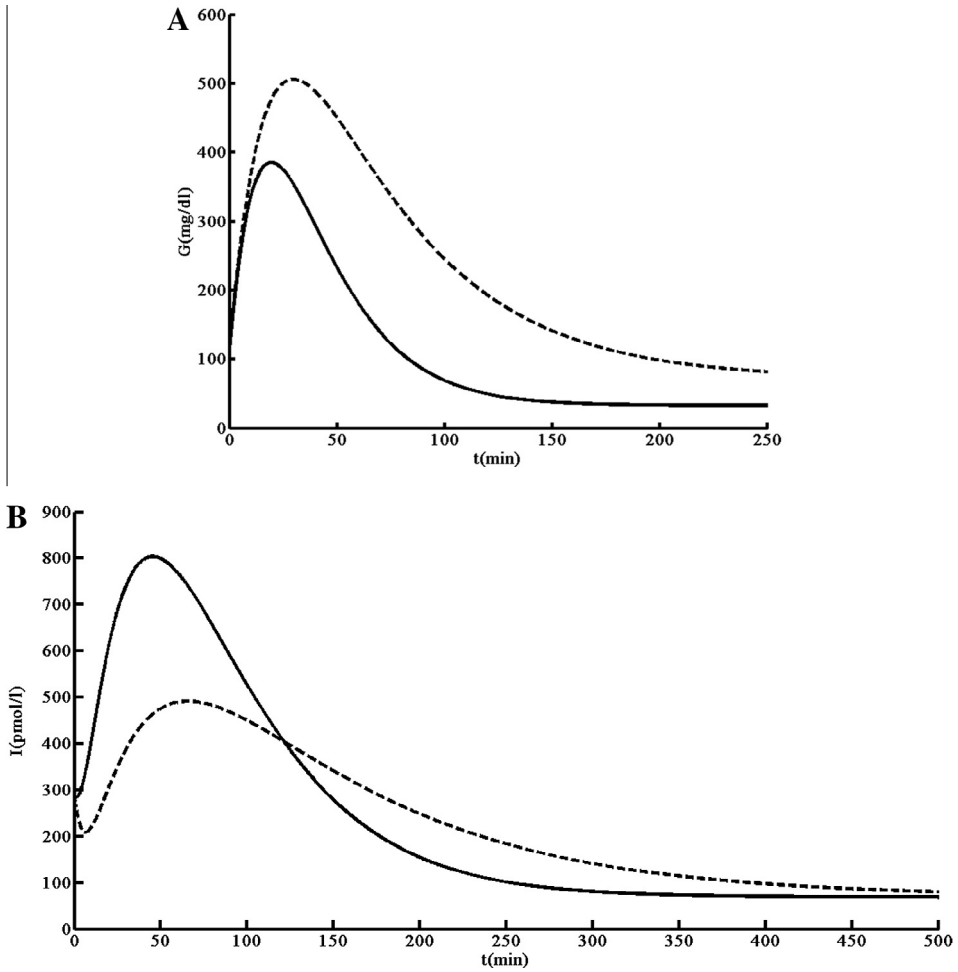

Fig. 5. Simulation with different values of k_1 parameter. The graph (A) shows the simulation of blood glucose levels as a function of time and the graph (B) blood insulin levels as a function of time. Full line is used when displaying graphs obtained with estimated and optimized values of k_1 for healthy rats, and dotted line with lower values of k_1.

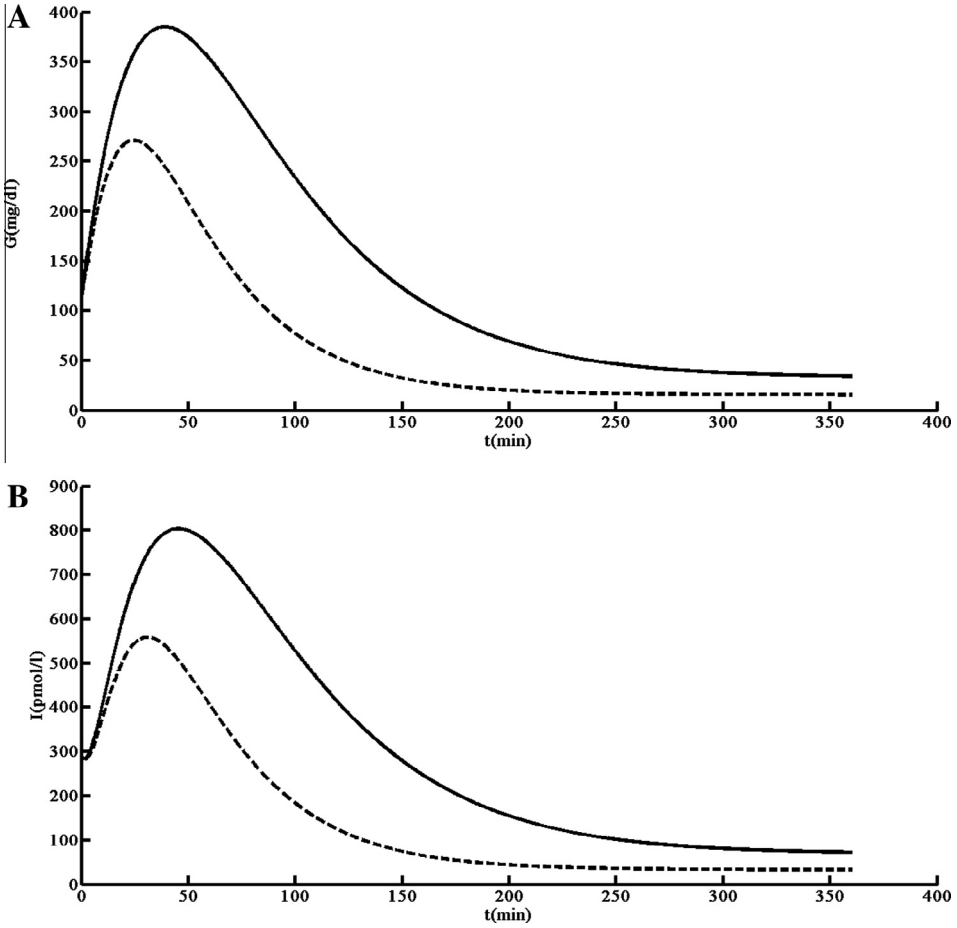

Fig. 6. Simulation with different values of k_2 parameter. The graph A shows the simulation of blood glucose levels as a function of time and the graph B blood insulin levels as a function of time. Full line was used when displaying graphs obtained with estimated and optimized values of k_2 for healthy rats, and dotted line when k_2 was increased.

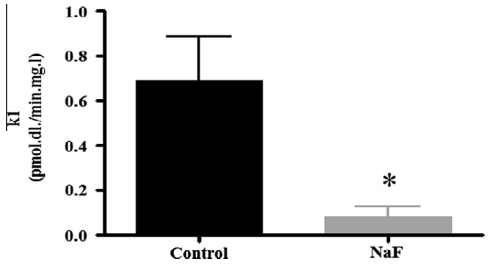

Fig. 7. Values of k_1 in control and NaF group. The plot shows the optimized values of parameter k_1 in Control (healthy rats) and NaF (with decreased insulin secretion) groups. Data are shown as mean ± SE and $*$ indicates significant differences from normal rats. $p < 0.05$ unpaired Student's t test.

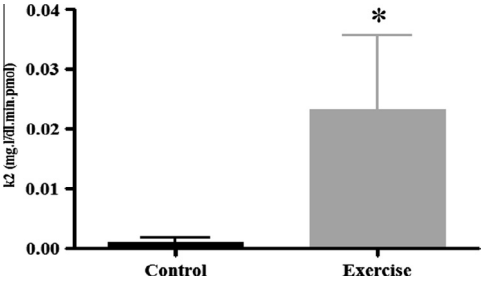

Fig. 8. Values of parameter k_2 in Control (sedentary rats) and Exercise groups. The plot shows the optimized values of parameter k_2 in Control (healthy rats) and Exercise (with increased insulin-dependent glucose uptake) groups. Data are shown as mean ± SE and $*$ indicates significant differences from normal rats. $p < 0.05$ unpaired Student's t test.

parameter with the model described in this paper. In the same animals the parameter was calculated as the constant of blood insulin clearance after an intravenous injection of insulin. Blood insulin levels were fitted with a one phase exponential decay, $I = I_0 * e^{-k_6 t}$. The values of k_6 obtained by the two methodologies were compared with paired Student's test.

No significant differences were found between the estimated and optimized values of k_6 ($n = 4$) using the mathematical model (0.1759 ± 0.1482) min^{-1} compared with the values obtained by nonlinear fitting as described after intravenous insulin injection (0.1323 ± 0.1038) min^{-1}. Paired Student's t test, $p > 0.05$

7. Discussion and conclusions

According to the World Health Organization there are over 220 million people in the world with diabetes and 3.4 million people died in 2004 as a consequence of this pathology. It is expected that deaths from diabetes will multiply by two between 2005 and 2030. This high incidence of diabetes worldwide and the significant deterioration in the quality of life experienced by the patient with diabetes has led to propose different strategies to treat this disease. The development of an artificial pancreas would restore the control

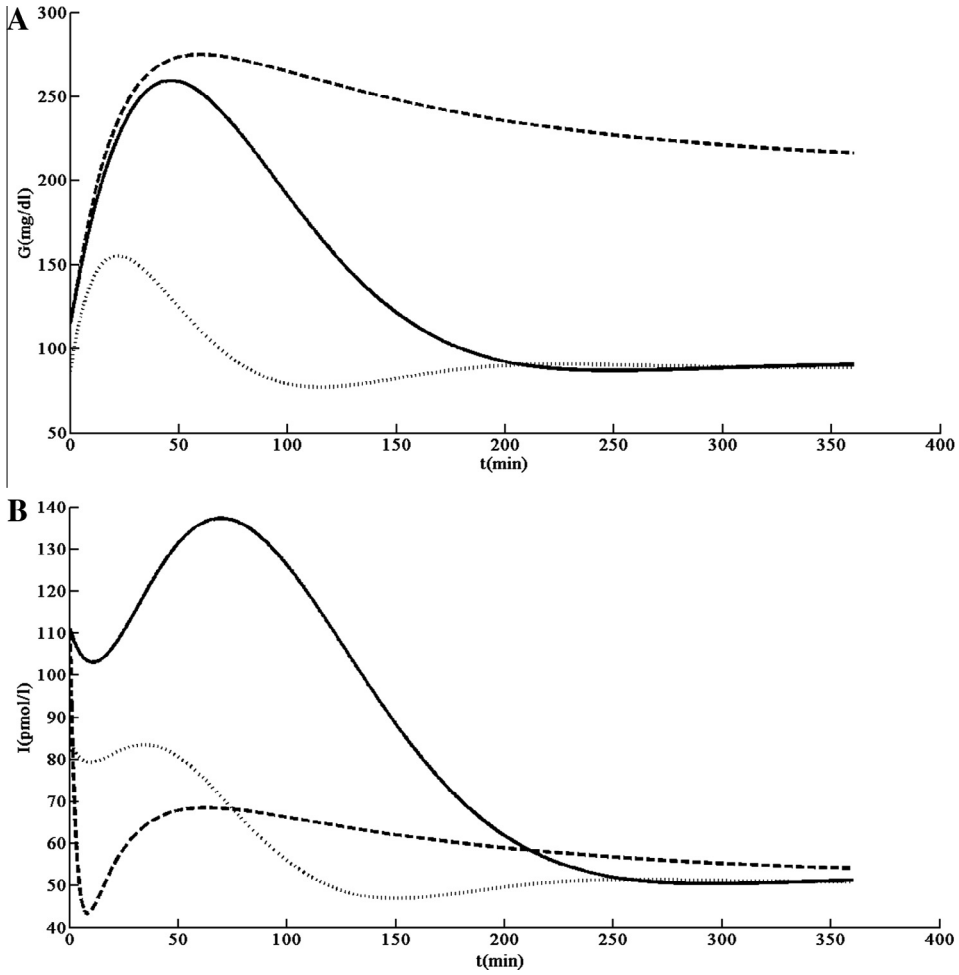

Fig. 9. Blood glucose and insulin in Control, NaF and Exercise groups. These graphs show the simulation curves of the three biological conditions: Control group (healthy rats) in solid line, NaF group (with decreased insulin secretion) in dashed-line and Exercise group (with increased consumption of glucose insulin-dependent) dot line. The graph A corresponds to the curves of blood glucose levels and the graph B to the curves of blood insulin levels.

of blood glucose by coupling an infusion pump to a continuous glucose sensor in blood. The design of such a device requires the development and application of mathematical models representing the gluco-regulatory system. Models developed by other research groups [1–5] as Cobeli's model, [12,13] describe very well the gluco-regulatory system but have a large number of mathematical equations and require complex methodologies for the estimation of its parameters. This model has 35 parameters, 12 differential equations and 18 algebraic equations. It is appropriate for the development of a simulator, but the high number of parameter makes it inappropriate for the application to individual cases. They are also developed for humans and the efforts to obtain all the parameters in the rat have been unsuccessful [14]. This was one of the reasons for developing a new model. This model was built to find a relationship between plasma glucose levels and insulin levels. In this manner, it would be useful for the development of a control algorithm that could be applied to an artificial pancreas.

This new simplified mathematical model of the glucose–insulin system for healthy rats presents, as opposed to other models, a small number of parameters that can be estimated by simple determinations of plasma glucose and insulin for each individual. The minimal model of Bergman, as ours, is a simplified model based upon physiology that could account for the glucose-insulin system; it has 3 differential equations and 7 parameters. All the parameters of the model can be estimated from a single data set using simple mathematical techniques and thus, avoiding unverifiable assumptions [15].However, the Bergman's model is a clinical tool to understand the composite effects of insulin secretion and insulin sensitivity on glucose tolerance and risk for type 2 diabetes mellitus. The model presented in this paper does not predict risk of diabetes but can represent and allow studying glucose homeostasis in different biological situations. It could be applied for any situation in which homeostasis of glucose insulin system is disturbed without the presence of glucose in urine (e.g. as described in the inhibition of insulin secretion by fluoride, this paper, Section 5.1 page 9). Also, this model has a parameter (k_2) that represents insulin sensitivity of the tissues and parameter (k_4) that represents the hepatic sensitivity to insulin. The values of these parameters are modified on biological situations where physiological processes are affected, as it was observed in the experiments with the exercise group (see Fig. 8, page 9).

Unlike other models, as Sturis, Polonsky, Tolic's or Li's model [16–18], this model does not include time delays to describe the circadian oscillation. However, in certain cases the sinusoidal behavior was observed. In this model the oscillation depends on the values of the parameters (see Fig. 2B, page 5).

In this paper, the proposed model has been validated in two different biological situations: experimentally decreased insulin production by the administration of NaF, and increased peripheral consumption of glucose by physical activity. The model parameters

were modified in the sense envisaged by the experimental intervention on carbohydrate metabolism. Another validation *in vivo* was made estimating the k_6 parameter using two methodologies: the model described in this paper and the plasma clearance of insulin after an intravenous injection. The obtained k_6 values did not differ significantly. In addition, we have shown that this model has a single biologically consistent equilibrium point. Using computer tools we could simulate variations in blood glucose and insulin generated by different intakes of glucose, as well as respond to changes in its parameters as expected. So far this model has been applied to healthy rats, but the immediate goal is to adapt it and validate it *in vivo* and *in silico* models for diabetic animals. This model might be a helpful tool for the experimental design of diabetes drugs research. Also, it enables the design and development of mechanisms for the control of glycaemia in order to improve the quality of life of diabetic patients.

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Appendix A.

A.A. Animals

Experiments were carried out in female Sprague Dawley rats of 200 ± 20 g body weight, fed with balanced food (GEPSA, Pilar, Córdoba, Argentina) and tap water *ad libitum*. The animal room had a dark/light cycle of 12 h/12 h and temperature of 23 ± 1 °C. Blood samples were obtained from the vein of the tail in heparinized tubes; they were centrifuged and plasma was saved at 20 °C to measure glucose and insulin concentrations. All experiments were performed in accordance with the international ethical guidelines of animal care [19,20]. The protocol was approved by the Ethics Committee, School of Medicine, Rosario National University.

A.B. Glucose oral administration

Animals with 8 h of fast received glucose (0,6 g/100 g body weight) by orogastric tube. Blood samples were obtained before and after glucose intake (0, 5, 10, 15, 30, 60, 90, 120, 180, 240, 300, 360 min).

A.C. Intravenous injection

Intravenous injection was applied for insulin (regular porcine insulin Betasint, Laboratorios beta SA. Buenos Aires, Argentina) injection and was performed through the lateral vein of the tail after disinfecting the area with alcohol [21]. A sterile 27G disposable needle was used. Blood samples were obtained before injection and after 30, 90, and 180 min.

A.D. Glucose Measurement

Glucose concentration was spectrophotometrically measured with a commercial kit (Wiener Laboratorios, Rosario, Argentina) in a Perkin Elmer lambda 11 spectrophotometer.

A.E. Insulin measurement

Measurement of blood insulin levels were carried out by RIA using a commercial kit (Ria kit Rat insulin, Millipore Corporation, Billerica, MA, USA).

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