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In vivo measurement of the rate constant of liver handling of glucose and glucose uptake by insulin-dependent tissues, using a mathematical model for glucose homeostasis in diabetic rats

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ABSTRACT

Diabetes mellitus is a disease that affects glucose homeostasis. The World Health Organization informs that there are over 347 million people in the world with diabetes. The diagnosis and characterization of glucose homeostasis in different metabolic conditions are subjects of great importance with high clinical impact. There are many mathematical models that describe the glucoregulatory system in detail. However, the use of these models is limited because they have a large number of mathematical equations and parameters and they require complex methodologies to estimate of them. This forced to work with average values that decrease the validity of results and the applicability of the models. In this study two mathematical models for rats with diabetes mellitus were developed. The difference between these models and others lies in the possibility of obtaining all parameters for each animal from simple measurements (glucose and insulin plasma levels). Moreover, the models allow to measure *in vivo* the different physiological processes involved in glucose homeostasis in animals: insulin secretion and its plasma clearance, absorption of insulin from a subcutaneous injection, the liver handling of glucose, intestine absorption of glucose, glucose uptake rate of insulin-independent tissues, glucose uptake rate of insulin-dependent tissues, and renal glucose excretion.

1. Introduction

Biological control of plasma glucose level involves several factors and tissues. One of the main factors is insulin. When this hormone fails to control plasma glucose levels, a disease known as diabetes mellitus arises. There are two types of diabetes mellitus: diabetes mellitus type 1 (DMT1), where insulin secretion is decreased, and diabetes mellitus type 2 (DMT2), where tissues are deficient in insulin response.

Usually, the fasting plasma level of glucose is measured to evaluate the glucose homeostasis. Sometimes, normal values of plasma glucose are the consequence of high insulin plasma level. Moreover, high values of insulin could be associated to high insulin pancreatic production or low insulin clearance. Thus, a method for evaluating insulin secretion and clearance separately would be very important and useful.

There are many indexes for the evaluation of insulin resistance: OGIS (Mari et al., 2001), homeostasis model assessment of insulin resistance (HOMA-IR) (Matthews et al., 1985), quantitative insulin sensitivity check index (QUICKI) (Katz et al., 2000), Matsuda index (Matsuda and DeFronzo, 1999), Avignon index (Pisprasert et al., 2013), Stumvoll index (Stumvoll et al., 2000). These indexes can be obtained from fasting glycaemia and fasting plasma insulin level, from data obtained after an oral glucose tolerance test (OGTT) or after an intravenous glucose tolerance test. However, there are not enough tests for measuring insulin secretion. Hyperglycaemic clamp (DeFronzo et al., 1979), intravenous glucose tolerance test (Pacini et al., 2013), and HOMA index (Matthews et al., 1985), allow the measurement of β-cell function. Nevertheless, the first two techniques need invasive and complex processes involving a risk for animals (Pacini et al., 2013). In addition, hyperglycaemic clamp needs anaesthesia that usually disturbs glucose metabolism

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(Behdad et al., 2014; De Oliveira et al., 2013; Xiao et al., 2013). Intravenous injection of glucose is challenging in rats without sedation or anaesthesia. HOMA index can be used to measure β-cell function from fasting glycaemia and fasting plasma insulin level through a computer model (Levy et al., 1998) using the software HOMA Calculator v.2.2 (Diabetes trials Unit, University of Oxford, available from http:/www.dtu.ox.ac.uk/homacalculator/index.php). Although this index can be calculated easily, it only measures insulin resistance and β-cell function, and it can be calculated with glycaemia between 50–500 mg/dl and plasma insulin level between 20–400 pmol/l. As a consequence HOMA index can be used in human beings, however, lower or higher plasma glucose and insulin levels are frequently found in rats, which difficult the use of HOMA index (Di Loreto and Rigalli, 2009).

Moreover, these methods are not able to measure other physiological processes involved in plasma glucose control such as liver handling of glucose or glucose uptake rate of insulin-independent tissues, glucose uptake rate of insulin-dependent tissues, and renal glucose excretion, and absorption of insulin from a subcutaneous injection (when insulin is administered).

In a previous work, a mathematical model of glucose-insulin system (System 1) and the methodology for the estimation and optimization of all the parameters were developed (Lombarte et al., 2013). This model was validated through *in vivo* experiments which demonstrated that the model represents adequately the changes of plasma glucose and insulin levels through time. Moreover, the model was used to evaluate changes in glucose homeostasis in other studies (Brenner et al., 2014; Lombarte et al., 2016). The model consists of 3 differential equations that represent the variation of plasma insulin levels (Eq. (1)), plasma glucose levels (Eq. (2)), and glucose amount in the digestive system (Eq. (3)).

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

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

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

System 1. Mathematical model for healthy rats.

Eq. (1) includes parameters that represent secretion (k_1) and plasma clearance of insulin (k_6). Eq. (2) includes parameters associated to liver function (k_4, I_{pi}), intestinal absorption ($k_{0,}$), insulin dependent tissues (k_2) and insulin independent tissues (k_3) activities. Finally, Eq. 3 includes the amount of glucose in the digestive system (D) and the rate constant of glucose absorption (k_a).

In the current study, two mathematical models for diabetic rats were developed based on the model described previously; and used to measure *in vivo* the different physiological processes involved in glucose homeostasis in animals with different metabolic conditions.

2. Model for rats with DMT2

2.1. Model formulation

Fig. 1 shows a representative diagram of the biological model used for the development of the mathematical model for rats with DMT2. Unlike the model for healthy rats, the present model includes the kidney and urine, where glucose is excreted. This compartment was include due to diabetic subjects excrete glucose in urine when plasma glucose level is higher than a threshold value (Bales et al., 1984).

The model for DMT2 have 4 differential equations that represent variations of plasma glucose level (G) and plasma insulin level (I), amount of glucose in the digestive system (D) and amount of glucose in urine (U), (System 2).

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

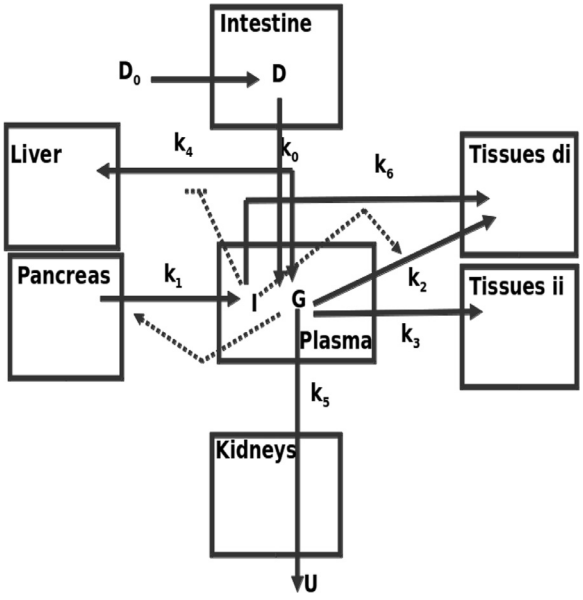

Fig. 1. Model for rats with DMT2.
Solid lines represent flow of glucose or insulin, dotted lines represent stimulatory (arrowhead) or inhibitory (blunt) effect. G: plasma glucose level (mg/dl), I: plasma insulin level (pmol/l), D: amount of glucose in the intestine (mg), U: amount of glucose in urine (mg). D_0: amount of glucose incorporated by the diet (mg), k_0: plasma glucose uptake from diet constant ($dl^{-1}.min^{-1}$), k_1: production of pancreatic insulin rate constant (pmol.dl/min.mg.l), k_2: rate constant of glucose uptake in insulin-dependent tissues, tissues di (mg.l/dl.min.pmol), k_3: rate constant of glucose uptake in insulin-independent tissues, tissues ii (mg/min.dl), k_4: uptake constant (for glycogenesis) or glucose release (by glycogenolysis and/or gluconeogenesis) by the liver (mg.l/dl.min.pmol), k_5: rate constant of glucose renal excretion (min^{-1}), k_6: plasma clearance of insulin (min^{-1}).

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

$$dG/dt = k_0 D - k_2 I - k_3 - k_4(I - I_{pi}) - k_5(G - G_u)H \tag{4}$$

$$H(G):$$
$$H = 1 si G > G_u \tag{5}$$
$$H = 0 si G \leqslant G_u$$

$$dU/dt = k_5(G - G_u)H \tag{6}$$

System 2. Mathematical model for rats with DMT2.

Eq. (1) represents variation of plasma insulin level. The term $k_1 G$ represents pancreatic insulin secretion, which is regulated by plasma glucose level; and the $-k_6 I$ term represents plasma clearance of insulin.

Eq. (3) represents variation of glucose in the intestine and the k_a parameter is the rate constant of glucose absorption.

Eq. (4) represents variation of plasma glucose level. The term $-k_4(I-I_{pi})$ represents hepatic handling of glucose. It is a positive term when plasma insulin level is lower than I_{pi} (indicating the contribution of glucose to plasma by glycogenolysis and gluconeogenesis) and it is a negative term when plasma insulin level is higher than I_{pi} (indicating the glucose uptake of glucose by liver for glycogenogenesis, glycolysis, or synthesis of lipids). I_{pi} is a parameter that represents plasma insulin level when the liver changes from glucose uptake to production. The $-k_3$ term represents glucose uptake by insulin-independent tissues. The $-k_2 I$ term represents glucose uptake by insulin-dependent tissues and $k_0 D$ the variation of plasma glucose level due to oral glucose administration. The $-k_5(G-G_u)H$ term represents renal excretion of glucose. G_u is the glucose renal threshold that is the value of plasma glucose level when renal transport of glucose is saturated and glucose begins to be excreted in urine. H is a function of glycaemia ($H(G)$)

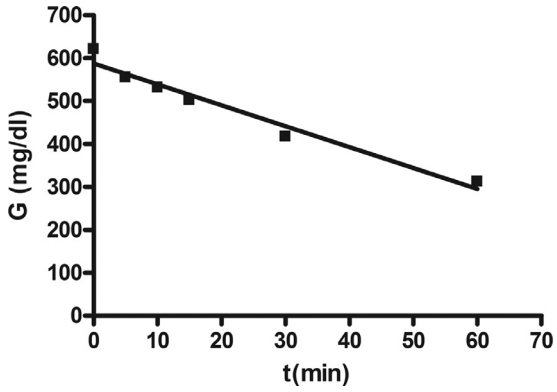

Fig. 2. Plasma glucose level as a function of time on a fated rat with diabetes. Squares points represent plasma glucose level. Continuous line represents the fit obtained with Eq. (7).

and it is defined by sections (Eq. (5)) in order to represent renal excretion of glucose. When plasma glucose level is lower than G_u, H is zero and there is not renal glucose excretion. In contrast, when plasma glucose level is higher than G_u, H is 1 and the renal glucose excretion depends on the difference between G and G_u.

Eq. (6) represents variation of glucose in urine, where the k_5 parameter is the rate constant of glucose urine excretion.

2.2. Estimation of parameters

All parameters were estimated by fitting different functions to the values of plasma glucose and/or insulin obtained after different experiments.

2.2.1. Estimation of k_a, k_0, k_1, k_2, k_3, k_4, k_6 and i_{pi}

These parameters were estimated using an OGTT. Animals received a glucose dose (0.3 g/100 g body weight) by orogastric tube (Lupo et al., 2009), after 8 h of fasting. Blood samples were obtained before (0 min) and after glucose administration (5, 10, 15, 30, 60, 90, 120, 180, 240, 300, 360 min). Plasma glucose and insulin levels were measured as stated in the appendix. k_a, k_0, k_1, k_2, k_3, k_4, k_6 and I_{pi} parameters were estimated from glucose and insulin plasma levels using a script developed for R environment that was made using the methodology developed for healthy rat (Lombarte et al., 2013). This script could be downloaded from http://hdl.handle.net/2133/10176 or http://www.biologiaosea.com.ar/software.html.

2.2.2. Estimation of k_5 and G_u

The k_5 and G_u parameters were estimated from glucose levels in urine and plasma. Animals were placed in metabolic cages with water *ad libitum* and without food. Urine and blood were collected at definite time intervals. Subsequently, glucose levels in plasma and urine were measured and values were used to make the adjustments described below.

In fasting diabetic rats, plasma glucose levels decrease linearly; Fig. 2 shows representative values of one rat.

Thus, glucose can be adjusted with Eq. 7 (Fig. 2).

$$G = G_0 - k_f t \tag{7}$$

Eq. (6) represents the variation of urinary glucose excretion as a function of the difference between G and the threshold glucose (G_u).

$$dU/dt = k_{5'}(G - G_u) \tag{6a}$$

Replacing G in Eq. (6), by Eq. (7), and integrating with respect to time:

$$\int dU = \int k_{5'}\left(G_0 - k_f t\right)dt - \int k_{5'} G_u dt \tag{8}$$

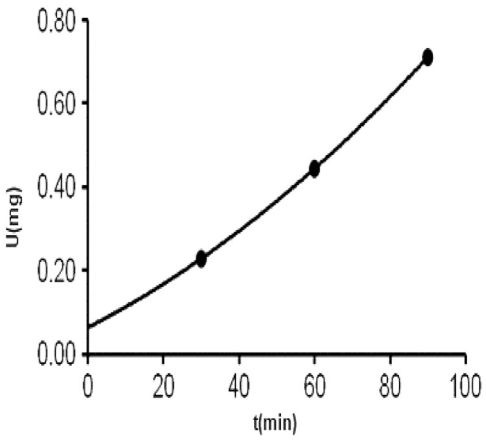

Fig. 3. Estimation of k_5 and G_u parameters. The dots represent the values of the amount of glucose (mg) in urine. The solid line represents the adjustment made with Eq. (12).

$$U = \int k_{5'} G_0 dt - \int k_{5'} k_f t dt - \int k_{5'} G_u dt \tag{9}$$

$$U = k_{5'} G_0 t - \frac{k_{5'} k_f}{2} t^2 - k_{5'} G_u t + c \tag{10}$$

$$U = k_{5'}(G_0 - G_U)t - \frac{k_{5'} k_f}{2} t^2 + c \tag{11}$$

The urinary excretion of glucose (U) through time (t) is a quadratic function of time with coefficients a, b and c (Eq. (12)), these coefficients are obtained by fitting experimental values with Eq. (12) (Fig. 3).

$$U = bt - at^2 + c \tag{12}$$

$$a = \frac{k_{5'} k_f}{2} \tag{13}$$

$$b = k_{5'}(G_0 - G_u) \tag{14}$$

From Eqs. (13) and (14) k_5 and G_u were obtained as following:

$$k_{5'} = 2ak_f \tag{15}$$

$$G_u = G_0 - bk_{5'} \tag{16}$$

$$G_u = G_0 - bk_f 2a \tag{17}$$

In these equations, G_0 is the plasma glucose level at the beginning of the fasting state, a and b are parameters of the non-linear fitting and k_f the slope of the linear function, which fits plasma glucose levels as function of time.

Fig. 3 shows the fitting using Eq. (12) that allows to obtain the values of a and b that are then used to obtain the parameters G_u and k_5.

2.3. Parameter optimization

Parameters were estimated in rats IIMβ/Fm. This strain of rats develops DMT2 in adult life (Calderari et al., 1989, 1987). Five male-adult rats (>300 days), body weight: 422.4 ± 31.4 g, were used. Estimated parameters were used as initial values for the optimization process using the Simulink tool of MatLab software.

In Fig. 4 measured insulin and glucose plasma levels are shown, with the curves obtained from the model after the parameters optimization process. The proximity of the curve to the measured values indicates that the model reproduced properly the behaviour of plasma glucose and insulin levels.

Table 1 shows the values of the optimized parameters of each rat of DMT2 group.

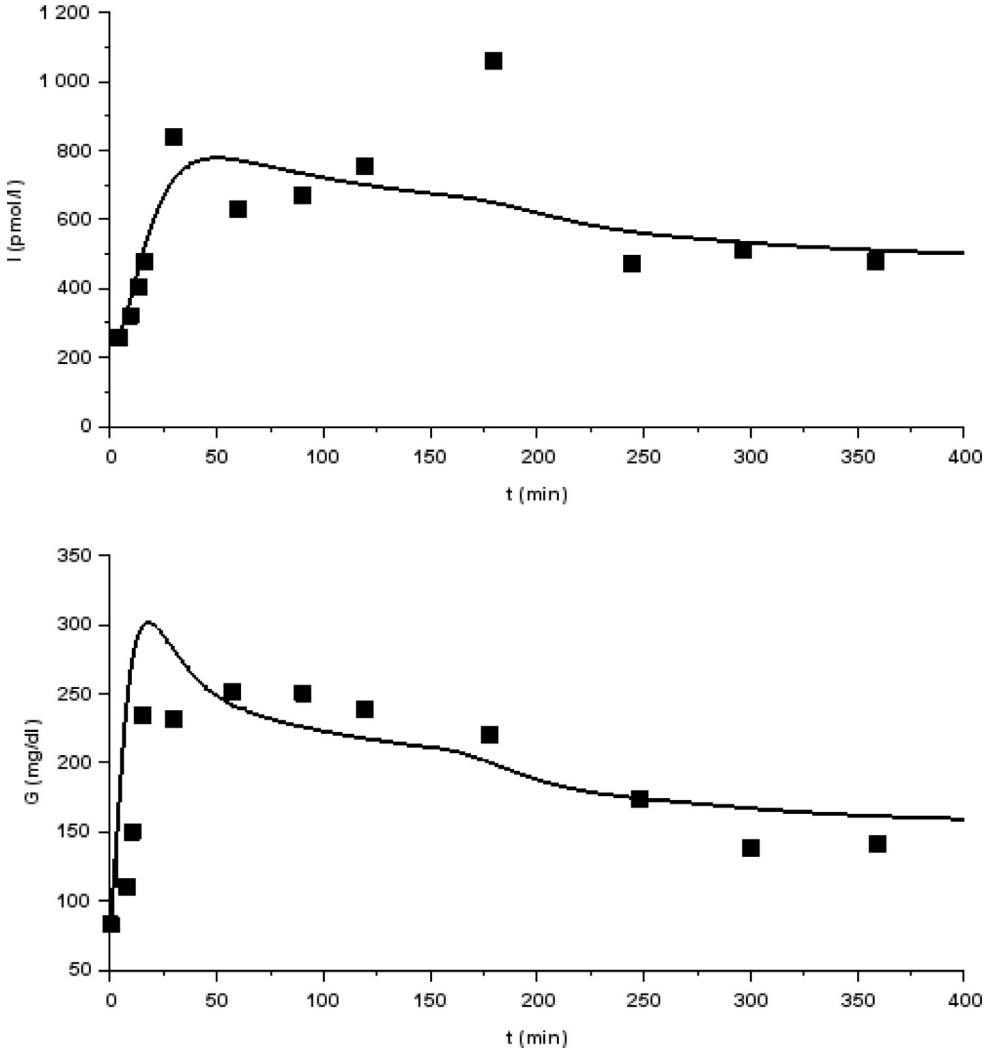

Fig. 4. Parameters optimization curves in a rat with DMT2.
Plots show the plasma Insulin level (top) and plasma glucose level (bottom) in a rat from DMT2 group after an intake of glucose. The squares points represent the measured values of plasma insulin level and plasma glucose level; and the continuous lines the curves obtained from the optimization process.

Table 1
Parameters optimized of rats with DMT2.

G_u	I_{pi}	k_0	k_1	k_2	k_3	k_4	k_5	k_6	k_a
210.9	617.3	0.008	0.155	1.56E−003	1.911	0.020	0.150	0.050	0.010
232.0	1103.9	0.026	0.330	2.35E−004	2.342	0.008	0.258	0.052	0.060
447.9	1136.5	0.001	0.586	4.51E−005	1.907	0.185	1.402	0.144	0.003
320.3	1356.1	0.011	0.398	3.48E−004	1.496	0.016	1.163	0.094	0.025
213.8	1293.9	0.020	0.432	3.08E−005	19.928	0.011	0.135	0.059	0.002

3. Model for rats with DMT1

3.1. Model formulation

Fig. 5 shows a representative diagram of the biological model used for the development of the mathematical model for rats with DMT1. The present model includes three new compartments and/or organs respect to the model of healthy rats. These new compartments are: the subcutaneous compartment where insulin is injected, the kidneys, and urine where glucose is excreted.

In animals with DMT1, the insulin secretion is very low or null thus the k_1G term could be omitted. Therefore, animals need exogenous insulin treatment; usually insulin is administrated by subcutaneous injection. In order to include in the model this situation, a new term has been added to Eq. (18): k_7Y, where Y represents the amount of insulin in the subcutaneous compartment and k_7 the rate constant of incorporation of exogenous insulin to plasma. Furthermore, a new equation was introduced in the model (Eq. (19)), which represents the variation of insulin level in the subcutaneous compartment. The other equations are the same than in DMT2 model (Eqs. (3)–(6)).

$$dD/dt = -k_aD \tag{3b}$$

$$dG/dt = -k_4(I - I_{pi}) - k_3 - k_2I + k_0D - k_5(G - G_u)H \tag{4a}$$

$$H(G):$$
$$H = 1 si G > G_u \tag{5a}$$
$$H = 0 si G \le G_u$$

$$dU/dt = k_{5'}(G - G_u)H \tag{6b}$$

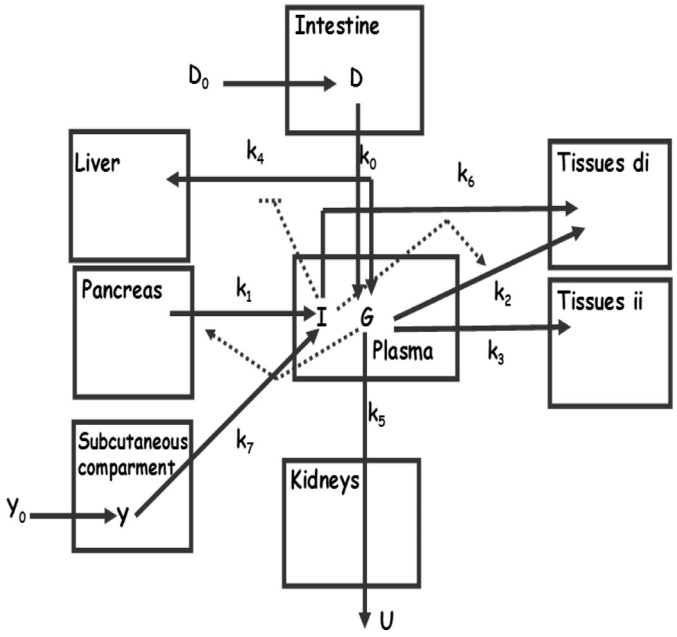

Fig. 5. Model for rats with DMT1.
Solid lines represent flows of glucose or insulin, dotted lines represent stimulatory (arrowhead) or inhibitory (blunt) effects. *G*: plasma glucose level (mg/dl), I: plasma insulin level (pmol/l), *D*: amount of glucose in digestive system (mg), *Y*: amount of insulin into subcutaneous compartment (UI), *U*: amount of glucose in urine (mg). D_0: amount of glucose incorporated from diet (mg), k_0: plasma glucose uptake constant from digestive system ($dl^{-1}.min^{-1}$), k_1: production rate of pancreatic insulin constant (pmol.dl/min.mg.l), k_2: rate constant of glucose uptake in insulin-dependent tissues, tissues di (mg.l/dl.min.pmol), k_3: rate constant of glucose uptake in insulin independent tissues, tissues ii (mg/min.dl), k_4: uptake constant (for glycogenesis) or glucose release (by glycogenolysis and/or gluconeogenesis) by liver (mg.l/dl.min.pmol), k_6: plasma disappearance of insulin constant (min^{-1}). Y_0: subcutaneous insulin dose (IU), k_7: rate constant of incorporation to plasma of exogenous insulin (pmol/l.min.UI), k_5: rate constant of glucose renal excretion (min^{-1}).

$$dI/dt = -k_6 I + k_1 G + k_7 Y \tag{18}$$

$$dY/dt = -k_8 Y \tag{19}$$

System 3. Mathematical model of rats with DMT1.

3.2. Parameter estimation

3.2.1. Estimation of k_a, k_0, k_1, k_2, k_3, k_4, k_6, I_{pi}, k_5 and G_U

These parameters are estimated in the same way as explained for DMT2 rats.

3.2.2. Estimation of k_6, k_7 and k_8

In order to estimate k_7 and k_8, a dose of long acting porcine insulin was subcutaneously injected (ISCI) and plasma glucose and insulin levels were measured for 6 h. Different fits from plasma glucose and insulin levels allow obtaining these parameters.

Estimation of k_8 and k_6

The parameter k_8 can be estimated using the residuals method (Gabrielsson et al., 2007). This method consists in: considering that after the maximum plasma insulin level, the processes that provide insulin to plasma are negligible compared to those that consume it. In this situation, insulin variation as function of time can be represented by Eq. (20). This differential equation can be solved to yield Eq. (21), where I_{MI} is the maximum insulin value reached during the experiment and k_6 the plasma clearance of insulin (due to its action in the tissues).

$$dI/dt = -k_6 I \tag{20}$$

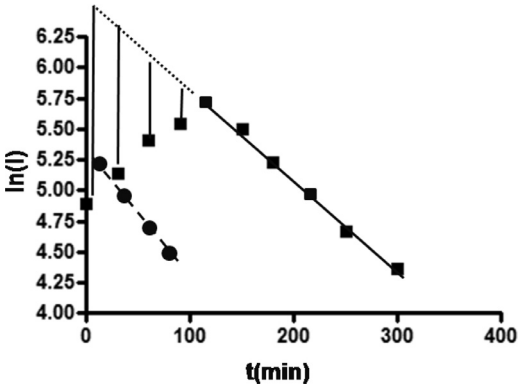

Fig. 6. Scheme for the residuals method used for calculation of k_8.
The black squares represent the logarithm of plasma insulin level after subcutaneous injection of insulin. The solid black line represents the fit using Eq. (22), which slope is k_6. The dotted black line represents the extrapolation of this function to times before insulin maximum (I_{MI}), black circles show the logarithms of residual insulin. The dashed line represents the second linear regression, which slope is k_8.

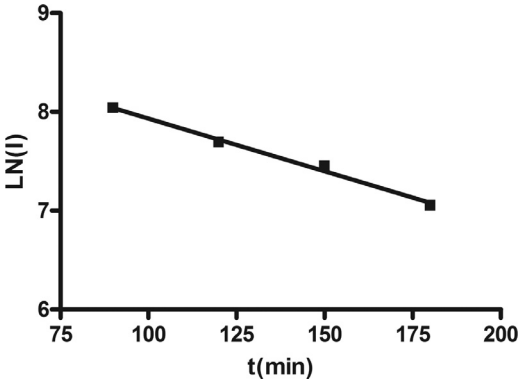

Fig. 7. Fit performed to estimate k_6.
The black squares represent the logarithm of plasma insulin levels after maximum insulin plasma level obtained with a subcutaneous injection of insulin. The solid black line represents the fit using Eq. (22), which slope is k_6.

$$I = I_{MI} e^{-k_6 t} \tag{21}$$

Applying logarithm on both members of **Eq. (21)**, **Eq. (22)** is obtained:

$$\ln I = \ln I_{MI} - k_6 t \tag{22}$$

Therefore, the slope of the plot of the logarithm of plasma insulin level as a function of time from its maximum value represents the plasma disappearance of insulin (k_6), (Fig. 6).

Using the regression parameters, the values of insulin for times before the I_{MI} could be calculated. Subtracting these values to the measured values of plasma insulin; the values that estimate residual insulin present in subcutaneous space are obtained. Finally, the linear regression of the logarithm of the residual insulin values *versus* time allows to obtain a straight line which slope represents the absorption rate of insulin from the subcutaneous space (k_8), (Fig. 6–8).

Estimation of k_7

A dose of insulin (Y_0) is injected to an 8 h fasted rat. Immediately after insulin injection, the processes that remove insulin from plasma are negligible with respect to those that provide insulin to plasma. For this reason we consider negligible the term $k_6 I$. This assumption is supported by significant increase in plasma insulin concentration. Pancreatic insulin secretion in animals with DMT1 is very low or null; therefore, the term ($k_1 G$) is negligible respect

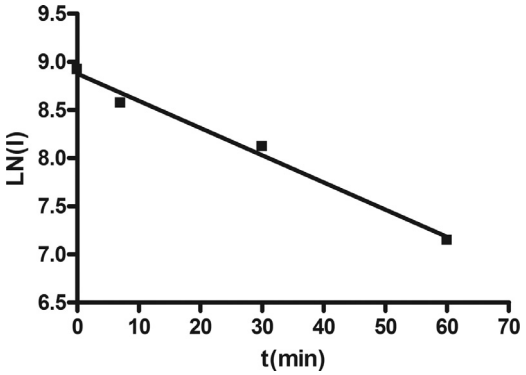

Fig. 8. Fit performed to estimate k_8.
The black squares represent the logarithm of residual insulin values after using the residual method. The solid black line represents the fit used to estimate k_8.

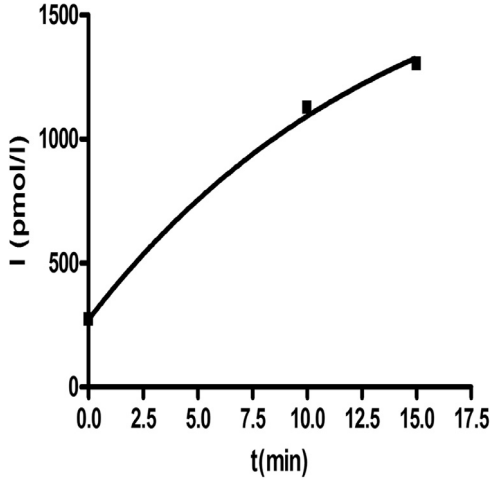

Fig. 9. Graph of the fit made to estimate k_7 parameter.
The black dots represent the values measured during the ISCI of insulin. The continue line represents the adjustment made for k_7 estimation (with Eq. (25)).

to plasma incorporation of exogenous insulin (k_7Y). According to these simplifications, the equation that represents the variation of plasma insulin Eq. (18)) could be reduced to Eq. ((23):

$$dI/dt = k_1G - k_6I + k_7Y \tag{18a}$$

$$dI/dt = k_7Y \tag{23}$$

$$Y = Y_0e^{-k_8t} \tag{19a}$$

Replacing Y with Eq. (19), which represents the content of insulin in the subcutaneous space, in Eq. (23), Eq. (24) is obtained:

$$dI/dt = k_7Y_0e^{-k_8t} \tag{24}$$

The solution of Eq. (24) represents plasma insulin levels as a function of time, for times close to the insulin injection (Eq. (25))

$$I = I_0 + \frac{k_7Y_0}{k_8}\left(1 - e^{-k_8t}\right) \tag{25}$$

k_7 is obtained by fitting the values of plasma insulin levels between 0 and 15 min after insulin injection with Eq. (25). k_8 is a parameter obtained using the method of the residual, Y_0 is the amount of insulin injected, and I_0 is plasma insulin level measured at the beginning of the experiment (time = 0 min). Fig. 9 shows plasma insulin levels used to calculate the parameter k_7.

3.3. Parameters optimization

Parameters were estimated in rats with DMT1 ($n = 7$) using the previously described methodology. In 70-days old female Sprague Dawley rats, body weight: 260.2 ± 40.6 g; DMT1 was induced through intraperitoneal injection of streptozotocin (60 mg/kg body weight dissolved in sodium citrate solution pH = 4.5) (Di Loreto and Rigalli, 2009). The disease development was assessed after 48 h of injection when fasting plasma glucose levels were higher than 200 mg/dl (376.4 ± 67.60 mg/dl), Mann Whitney test was used for a single sample, $p < .05$. Animals were treated with subcutaneous insulin in order to maintain plasma glucose values below 200 mg/dl. The insulin treatment was suspended 24 h before parameters estimation. The estimated parameters were used as initial values for the optimization process using the Simulink tool of MatLab software.

The parameters estimation in DMT1 rats was achieved by two experiments: OGTT and ISCI, thus two optimization processes were performed.

Fig. 10 shows the plasma levels of glucose and insulin obtained using the model for an animal with DMT1 and the values measured after OGTT.

Fig. 11 shows curves of the same variables (plasma levels of glucose and insulin) generated by the model after the optimization process compared to experimental values of plasma glucose and insulin after the ISCI. Plots obtained after the optimization process demonstrate that the proposed model produces glucose and insulin curves comparable to the measured values.

Table 2 shows the values of the optimized parameters of each rat of DMT1 group.

3.4. Numerical simulation

In order to understand the system behaviour, simulations were done using the MatLab Simulink library. Simulation plots were made with the values of the parameters estimated and optimized in rats, and then some theoretical modifications in parameters values were generated. In this paper we show data from simulation with two glucose intakes (1500 and 2500 mg), and with two doses of subcutaneous insulin injection (0.5 and 1 IU).

Fig. 12 shows the plots of plasma glucose and plasma insulin levels that were obtained from simulation after the intake of two different amounts of glucose. As expected, higher intakes of glucose produce higher plasma glucose levels.

Simulations of plasma glucose and insulin levels were made with two values of subcutaneous insulin (0.5 and 1 UI). In the case of a higher dose of insulin, higher values of plasma insulin and lower values of glycaemia were expected.

Plasma glucose and insulin levels obtained during the simulation are shown in Fig. 13.

3.5. Validation of the model: evaluation of the diagnostic capability of k_1 parameter to detect insulin secretion and comparison with HOMA-IR index

ROC analysis was performed in order to evaluate the diagnostic capacity of k_1 to detect low insulin secretion. This analysis was made employing k_1 parameter values obtained from Control group (healthy rats that have normal insulin secretion) and DMT1 group (rats with DMT1 that have low insulin secretion).

Simultaneously, ROC analysis was done with $\%\beta$ cell function calculated in Control and DMT1 group. ROC curve for k_1 and $\%\beta$ cell function were compared as detailed below.

The k_1 parameter value was significantly lower in DMT1 (0.0172 ± 0.006) group compared to Control group (0.1987 ± 0.059), Mann–Whitney $p < .05$.

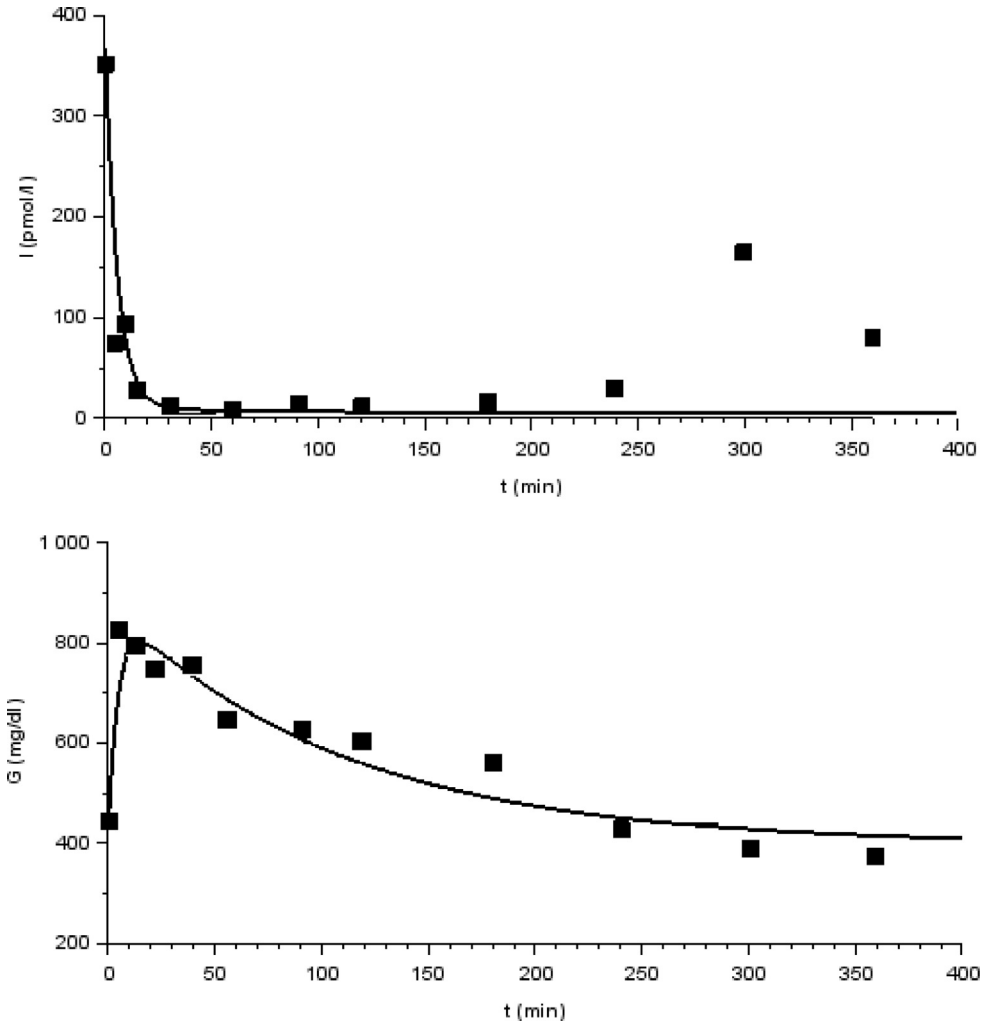

Fig. 10. Parameters optimization curves in a rat with DMT1 after an OGTT.
Plots show plasma Insulin levels (top) and plasma glucose levels (bottom) in a rat with DMT1 after an OGTT. The dots represent the measured values of plasma insulin and glucose levels; and the continuous line the curves obtained from the model.

Table 2
Optimized parameters in rats with DMTI.

G_u	I_{pi}	k_0	k_1	k_2	k_3	k_4	k_5	k_6	k_a
387.0	298.5	0.004	0.002	1.53E−05	3.543	0.127	0.047	0.002	0.007
350.6	125.2	2.130	0.024	0.0019	1.383	6.119	0.338	0.100	0.070
315.4	152.8	0.007	0.050	0.0747	12.314	0.277	0.013	0.092	11.12
220.6	129.5	0.031	0.025	0.0002	6.819	0.071	61.73	0.085	0.016
472.3	82.6	0.044	0.042	0.0061	3.010	0.058	0.914	0.102	0.023
190.1	18.7	3.326	0.001	0.3153	37.422	5.370	0.064	0.103	0.100
352.4	156.3	0.160	0.002	0.0067	2.244	0.017	0.009	0.170	0.206

The diagnostic capacity of k_1 parameter to determine low rate of insulin secretion was evaluated by ROC analysis. Fig. 14 shows the ROC curve for k_1 parameter. The area under the curve (AUC) was 1 with a confidence interval of 95%: 1–1, these values indicate that k_1 is a useful test for detecting low insulin secretion.

The threshold obtained for the k_1 parameter was 0.051 pmol.dl/l.min.mg. Therefore, rats with a k_1 value lower than 0.051 (units are omitted) has low insulin secretion. The diagnostic test has 100% of sensitivity and 100% of specificity. These values indicate that all rats with low insulin secretion are detected by the test and none rats with normal insulin secretion are considered with low insulin secretion. In other words, the test has no false negatives or false positives. Moreover, the test has a positive predictive value of 100%, this indicates that a k_1 value lower than the threshold represents, in 100% of the cases, rats with low insulin secretion. A value of k_1 higher than the threshold represents normal insulin secretion in 100% of the cases (negative predictive value = 100%).

ROC analysis of $\%\beta$ cell function shows a threshold of 70.7% and an AUC of 0.60 with a confidence interval of 95%: 0.362–0.8328. The diagnostic test has 90.9% of sensitivity and 50% of specificity. The ROC test shows that the ROC curve for k_1 was not different from the ROC curve for $\%\beta$ cell function ($p > .05$). These results indicate that k_1 parameter is as useful diagnostic test as $\%\beta$ cell function for the estimation of insulin secretion but has better sensitivity and specificity.

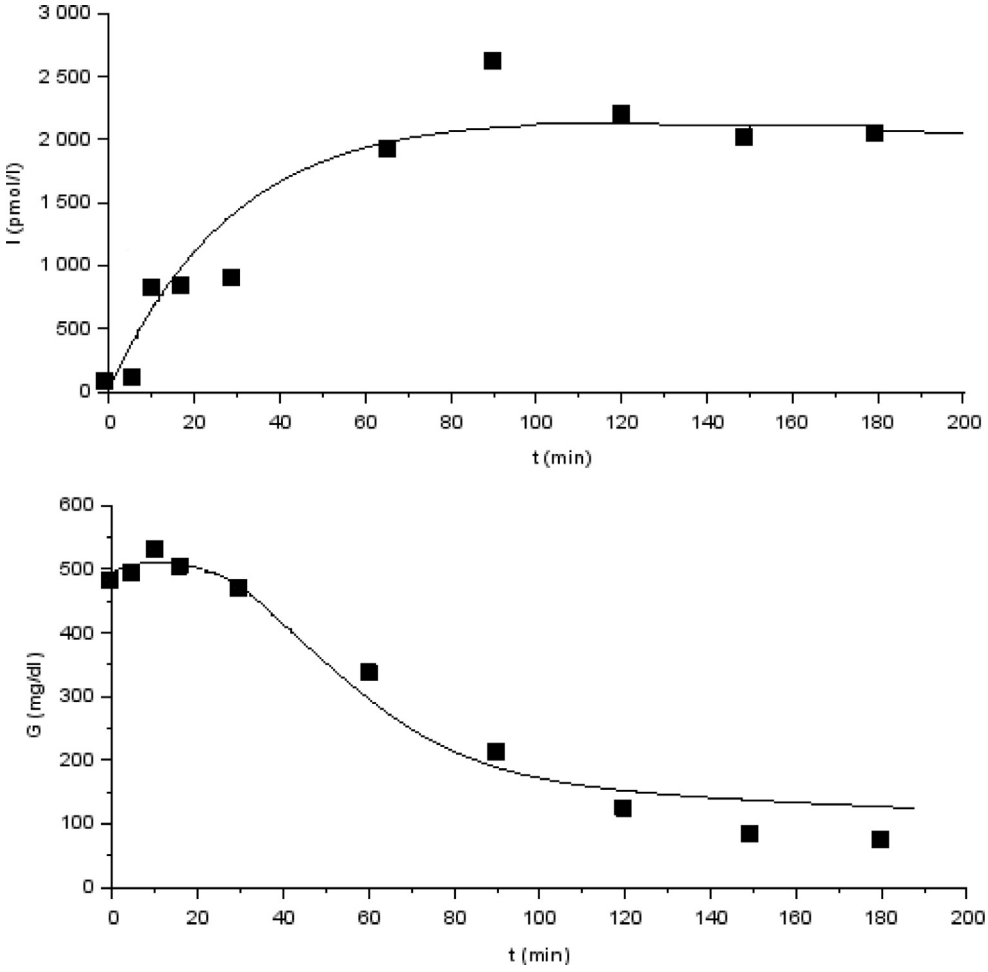

Fig. 11. Parameters optimization curves in a rat with DMT1 after an ISCI.
Plots show plasma Insulin levels (top) and plasma glucose levels (bottom) in a rat with DMT1 after an ISCI. The dots represent the measured data of insulin and glucose; and the continuous line the curves obtained with the model.

3.6. Application of the model: effect of fluoride in rate constant of different physiological processes involved in glucose homeostasis

The model for rats with DMT1 was used to measure the effect of fluoride in rate constant of different physiological processes involved in glucose homeostasis like: insulin secretion (k_1) and its plasma clearance (k_6), the liver handling of glucose (I_{pi}, k_4), intestine absorption (k_a and k_0), glucose uptake rate of insulin-independent tissues (k_3), glucose uptake rate of insulin-dependent tissues (k_2), and renal glucose excretion, G_u and k_5. Fluoride is a disturbing substance for the glucose-insulin system (Lombarte et al., 2015). Plasma glucose and insulin level were measured after an ISCI in animals with DMT1 treated with fluoride in drinking water. Data were obtained from an experiment conducted by researchers of the Department of Biochemistry, Faculty of Dentistry, University of Sao Paulo, Bauru, Brazil, with whom the Bone Biology Laboratory works in collaboration. In this experiment rats with DMT1 were treated with NaF in the drinking water (15 ppm) for 30 days. The DMT1 state was induced by injection of streptozotocin (Lobo et al., 2015). Parameters of the mathematical model were estimated in these rats and then these values were compared with values of the DMT1 group.

The studies performed in Brazil had demonstrated that fluoride increases insulin sensitivity in rats with DMT1 and that this increase is accompanied by changes in the expression of liver and muscle proteins (Leite et al., 2014). Therefore, we focus the attention on the k_2 parameter (insulin sensitivity), k_4 and I_{pi} (liver function parameters). Table 3 shows the median and range of these parameters. Fluoride treatment increased k_2 parameter which represents tissues insulin sensitivity (mainly skeletal muscle and adipose tissue), but this increase was not statistically significant different between groups. However, when assessing liver parameters a statistically significant increase was found in both parameters I_{pi} and k_4 (Table 3).

Table 3
k_2, k_4 and I_{pi} parameter values in rats with DMT1 treated with fluoride.
The median and range of parameter values k_2, k_4 and I_{pi} found in DMT1 rats treated with fluoride (15 ppm) in drinking water compared with the values found in DMT1 rats that did not receive fluoride (0 ppm). Different letters indicate statistically significant differences, Mann Whitney test $p < 0.05$.

Parameter	Fluoride	
	0ppm	15ppm
k_2	0.006 (1.5×10^{-5}–0.315)[a]	0.033 (0.014–0.165)[a]
k_4	0.127 (0.018–6.119)[a]	0.519 (0.000–0.635)[b]
I_{pi}	129.53 (18.76–298.52)[a]	267.68 (241.92–1143.70)[b]

4. Discussion and conclusions

A mathematical model of glucose-insulin system applicable to healthy rats was developed in a previous study (Lombarte et al., 2013). In the present research two new models for rats with DMT1

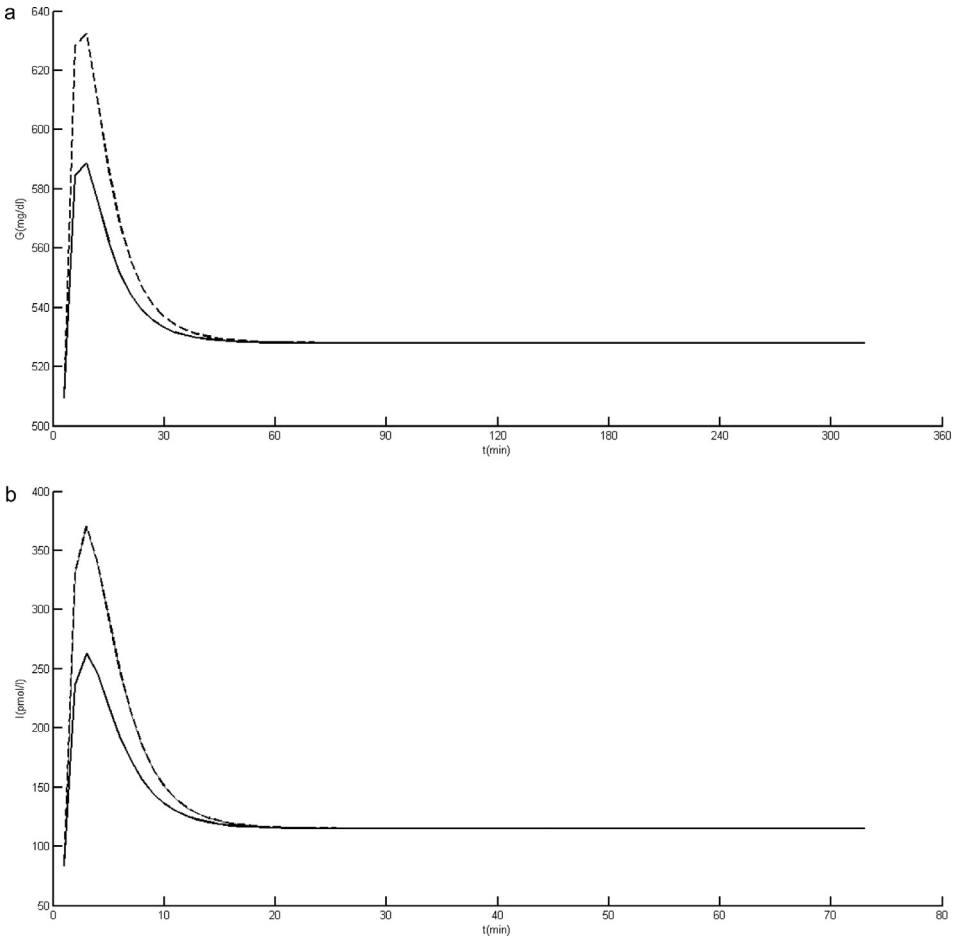

Fig. 12. Levels of plasma glucose (top) and plasma insulin (down) obtained during simulation with two different values of ingested glucose in a rat with DMT1. Solid lines represent de curves generated when a dose of 1500 mg glucose was administered and dashed line when the dose is increased to 2500 mg glucose.

and DMT2 were performed. There are lots of mathematical models that describe in detail the glucoregulatory system but they have a large number of mathematical equations and parameters; that require complex methodologies for their estimation (Bergman, 1970; Fabietti et al., 2006; Hovorka et al., 2004; Kovatchev et al., 2009; Sorensen, 1978). This forced to work with average values that decrease the validity of results and the applicability of the models. Unlike models developed by other researchers, the models described in this study have a reduced number of parameters. This feature allows the estimation of all the parameters of the model for each rat, using only plasma glucose and insulin measurements (common findings in clinical analysis laboratory of low complexity). This is a highlight advantage that could allow using the model in human beings. The *in silice* simulation showed that the model represents adequately the variations of glucose and insulin levels generated by the intake of different amounts of glucose and by different doses of insulin injection.

This study demonstrates that the value of the parameter k_1 is a good diagnostic test for the evaluation of insulin secretion in rats. The parameters of the models are easily calculated from glucose and insulin plasma levels employing a script developed by the authors. The proposed methodologies not only enable the measure of k_1 but also other parameters that represent the function of: liver, dependent and independent insulin tissues, etc.

The models proposed and the developed methodologies allow us to obtain all the parameters for each animal. Obtaining individual parameters for each rat avoids the use of average population values. The availability of individual parameter values for each rat allows quantifying each of the main processes of homeostatic process that involves the control of blood glucose in rats. In other studies the model was used to evaluate changes in processes involved in glucose homeostasis (Brenner et al., 2014; Lombarte et al., 2016).

The indisputable advantage of the mathematical models of this study over other models is the simplicity of the calculation and the ability to get all the parameters for a single individual. However, it has some disadvantages: (1) the model overestimates the values of plasma glucose level after 150 min in OGTT. This problem caught the attention of the authors and futures modifications of the models will be introduced; (2) the lack of in-depth description of some homeostatic processes. More complex models involve more detailed descriptions of these processes (Bergman, 1970; Fabietti et al., 2006; Hovorka et al., 2004; Kovatchev et al., 2009; Sorensen, 1978), but their usefulness is limited because they lack a mechanism for obtaining the parameter values in each individual application or require very expensive techniques that are not available in most of the diagnostic centres.

In this work, the new model for DMT1 rats was used to measure the effect of fluoride in DMT1 rats. The variation observed in the parameters of the model confirms the effects described in previous studies.

Thus, this work allows: to validate the use of mathematical modelling as a tool to study the different physiological processes involved in glucose-insulin homeostasis in individuals with different metabolic states, and to obtain a unique set of parameters for each individual, which may be used to develop strategies for

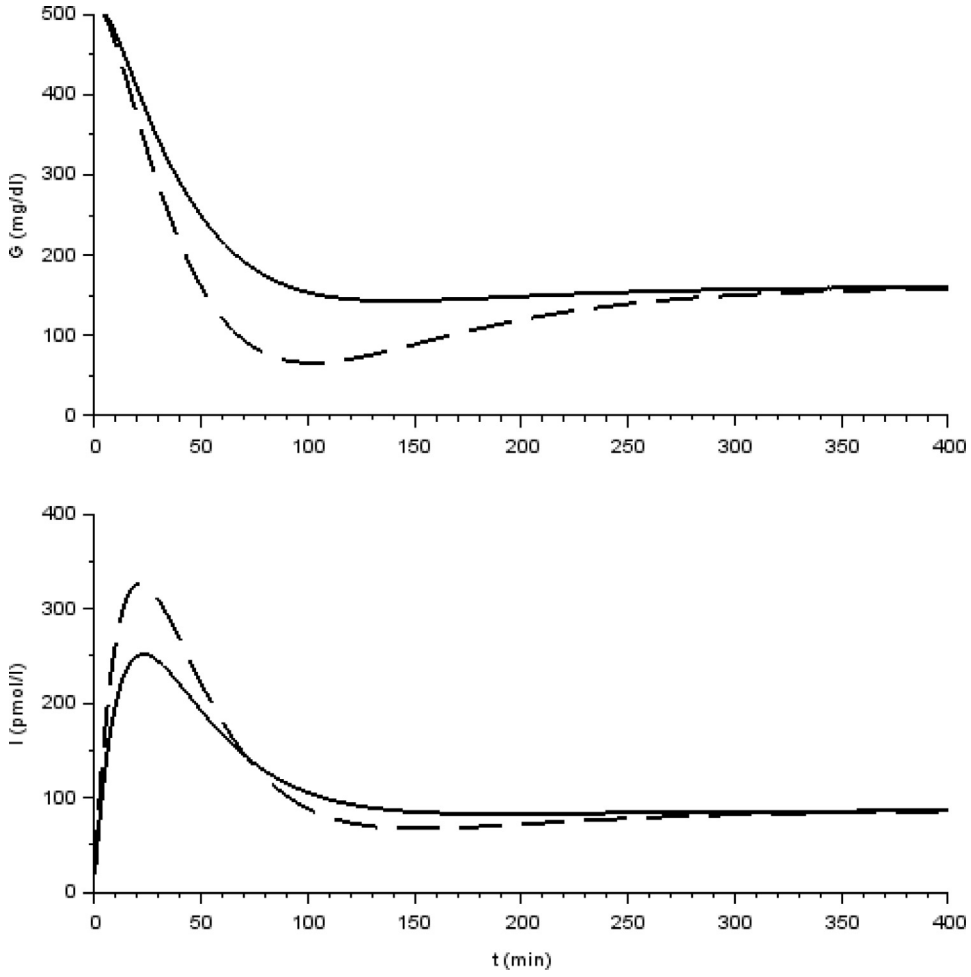

Fig. 13. Levels of plasma glucose (top) and plasma insulin (down) obtained during the simulation with two different values of insulin injected into a rat with DMT1. Solid lines represent de curves generated when a dose of 0.5 IU of insulin was administered and dashed line when the dose is increased to 1 IU of insulin.

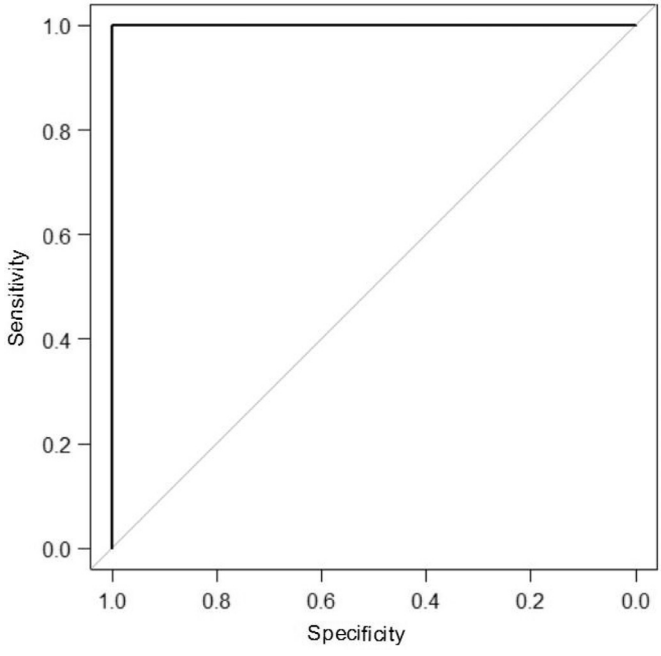

Fig. 14. Roc curve of k_1 parameter of Control and DMT1 groups (sensitivity vs specificity).
Area under de curve: 1, confidence interval of 95%: 1–1, threshold: 0.051 pmol.dl/l.min.mg, sensitivity: 100%, specificity: 100%, positive predictive value: 100% and negative predictive value: 100%.

glucose control using an insulin infusion pump. An infusion pump coupled to a continuous glucose sensor in blood constitutes an artificial pancreas. These devices would allow restoring control of blood glucose and they are an appropriate promising solution for diabetes treatment.

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APPENDIX

A. Animals

Experiments were carried out in Sprague Dawley and IIMβ/Fm rats, 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 (Olfert et al., 1993). The protocol was approved by the Ethics Committee, School of Medicine, Rosario National University.

B. Glucose oral administration (OGTT)

Animals with 8 h of fast received glucose (0.3 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).

C. Subcutaneous injection (ISCI)

Subcutaneous injection was applied for insulin (regular porcine insulin Betasint, Laboratorios beta SA. Buenos Aires, Argentina) and was performed in the abdominal area (Lupo et al., 2009) with a sterile 25 G disposable needle was used. Blood samples were obtained before injection and after 5, 10, 15, 30, 60, 90, 120, 180, 240, 300, 360 minutes.

D. Glucose measurement

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

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).

F. Statistic analysis

Data were expressed as median and range. Mann-Whitney test was used for comparisons of data between two groups. In all cases, differences were considered significant when $p < .05$.

ROC analysis was performed to evaluate the diagnostic ability of parameters k_1 and HOMA-IR, with the package pROC (Robin et al., 2011). All statistical analyses were performed with the computer programme R 2.14.1 (R Foundation for Statistical Computing. R, 2011).

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