Andrzej Kraslawski and Ilkka Turunen (Editors), Proceedings of the 23rd European Symposium on Computer Aided Process Engineering - ESCAPE 23, June 9 - 12, 2013, Lappeenranta, Finland

Scaling the Endocrine System from Rats to Humans

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Abstract

As a common laboratory practice rats are studied as biological models for understanding several human's diseases. This work is focused on studying the endocrine behavior to obtain a proper *in silico* healthy and diabetic rat which would be able to compare well with the experimental data. After this, the expectations are to get valuable insight to quantify the analogies with humans and to determine a realistic scale-up between both, humans and rats. With this purpose the scale-up procedure proposed in Hall et al. [2012] is taken into account. In this context, the well-known model of healthy subject given by Sorensen [1985] was proper to implement the scale-up methodology. Therefore, the main contribution of this work is to present the preliminary procedure of the scale-up focused on the specific problem of diabetes Mellitus. An intensive search in the literature had to be done to perform the simulations. Hence, several results are included and confronted with experimental data. These results will be useful to the pre-clinical testing of control algorithms with rats to be extrapolated to human beings.
Keywords: Endocrine System Model, Scale-Up, Rats, Humans, Experiments.

1. Introduction

Type I diabetes Mellitus disease is produced because the beta cells of the pancreas have been destroyed . These cells produce insulin, a hormone that regulates the blood glucose concentration in the body. As a common research methodology in this area, several pre-tests are done with rats having the possibility to infer the real effects in human beings. In this context, the rats represent valuable biological models to learn more about several human's diseases. This procedure has been useful for testing drugs in the endocrine system and understanding their mechanistic action in the body, aiding in therapy design, and serving as an accurate dosing tool. In the last three decades, the research community in this area has made important efforts to develop different mathematical models to represent the glucose-insulin interaction in healthy and diabetic humans. Particularly, in 2008 the US Food and Drug Administration approved a type I diabetic patient simulator as a substitute to animal trials in the pre-clinical testing of closed-loop control algorithms [Kovatchev et al., 2009] to formulate the basis for an artificial pancreas. It must be remarked that it recommends that human clinical test be preceded by at least one preclinical trial

on laboratory animal species providing substantial evidence for efficacy. In this context, we consider that having a mathematical model of the endocrine system for healthy and diabetic rats could be very useful to achieve good results in these directions. Therefore, a first approach was presented by Campetelli et al. [2010], where the mathematical model of the endocrine system developed by Kovatchev et al. [2009] for humans was adjusted to diabetic rats. In the work of Campetelli et al. [2010] the main purpose was to get insight about accurate scaling factors between humans and rats. Even though the obtained results were promising, it was difficult to do a scale-up because the model of Kovatchev et al. [2009] does not involve geometrical relations to be used. For this reason, we considered the first-principle model of Sorensen [1985] because it is the only physiologically based compartmental configuration which takes into account different parts of the human body such as brain, heart, lungs, liver, gut, kidneys and periphery. Hence, it represents a good starting point for testing the scaling procedure recommended in Hall et al. [2012]. So, in this paper the endocrine system model for healthy subject given by Sorensen [1985] is scaled for its use with healthy rats. The calculations done here are based on obtained data from an intense search concerning volumes and organs weights of rats together with some useful considerations reported in the literature and other based on experimental experience. The predictions of this scaled model for rats are confronted with the experimental data obtained in our laboratory. This allows to quantify the potentiality of our methodology and check if the scale-up is able to predict well the real impact observed through the experiments with rats to human beings. Hence, accurate models for rats together with a confident scaling factor will be helpful to speed up the knowledge gain from experimental trials and to address safety concerns. It must be noticed that a very detailed and complex computer model (cell level) of the progression of type 1 diabetes in the NOD mouse exists (Type 1 Diabetes PhysioLab®developed by Entelos in collaboration with the American Diabetes Association) but it is only commercially available.

2. The Endocrine System Model Framework and Scale-Up Methodology

In this section a summary of the model given by Sorensen [1985] together with the scale up method applied to estimate the parameters to be useful for mimic the rats endocrine system will be described. The original equations of the mathematical model given by Sorensen [1985] are detailed in the Appendix 4. It is a first principles model, has a physiological structure, is divided into two sub-compartments (interstitial fluid space and capillary spaces) where mass balances are derived. It was obtained from experimental evidence to formulate and validate metabolic processes of the compartmental model on the whole organ and tissue level, including glucagon as a counter-regulatory hormone. The glucose-insulin model is governed by 22 nonlinear ordinary differential equations and it is divided into three subsystems: Glucose, Insulin and Glucagon. The first two subsystems were modeled for the brain, arterial system (heart and lungs), liver, gut (stomach and intestines), kidney, and periphery (muscle and adipose tissue) compartments. The glucagon was modeled as a single blood pool compartment. Some typographical errors and inconsistencies in the differential equations have been reported elsewhere [Parker et al., 2000, Colmegna and Sánchez Peña, 2012] and here the corrections of Colmegna and Sánchez Peña [2012] were adopted. In this work, the rate of glucose appearance in blood (Γ_{meal}) was calculated using the model of Dalla Man et al. [2006] whose equations could be seen in Dalla Man et al. [2006]. It was assumed that rat organ masses had the same linear correlation as of humans. Analogously, the blood flow rates are assumed with the same

Table 1: Model Parameters

Parameter	Units	Rats	Humans
q_B	dl/min	0.0779	5.9
q_L	dl/min	0.1672	12.6
q_K	dl/min	0.1343	10.1
q_P	dl/min	0.2008	15.1
q_H	dl/min	0.7144	43.7
q_S	dl/min	0.1343	10.1
q_A	dl/min	0.0129	2.5
v_B^C	dl	0.0127	3.5
v_H^C	dl	0.0522	13.8
v_B^T	dl	0.0174	4.5
v_S^C	dl	0.0220	11.2
v_L^C	dl	0.0275	25.1
v_K^C	dl	0.0210	6.6
v_P^C	dl	0.0386	10.4
v_P^T	dl	0.2842	67.4
Q_B	l/min	0.0056	0.45
Q_L	l/min	0.0119	0.9
Q_K	l/min	0.0096	0.72
Q_P	l/min	0.0143	1.05
Q_H	l/min	0.0510	3.12
Q_S	l/min	0.0096	0.72
Q_A	l/min	0.0009	0.18
V_N	l	0.0473	9.93
F_{PNC}	l/min	0.0180	0.91
F_{PC}	Dimensionless	0.15	0.15
F_{KC}	Dimensionless	0.3	0.3
F_{LC}	Dimensionless	0.4	0.4
T_B	min	2.1	2.1
T_P^G	min	5	5
T_P^I	min	20	20

Parameter	Units	Rats	Humans
V_B^C	l	0.0009	0.265
V_H^C	l	0.0037	0.985
V_S^C	l	0.0016	0.945
V_L^C	l	0.0020	1.14
V_K^C	l	0.0015	0.505
V_P^C	l	0.0028	0.735
V_P^T	l	0.0284	6.3
T_I	min	25	25
T_R^I	min	65	65
G_L^B	mg/dl	101	101
G_{PI}^B	mg/dl	86.81	86.81
G_H^B	mg/dl	91.89	91.89
I_L^B	mU/l	21.43	21.43
I_{PI}^B	mU/l	5.304	5.304
I_H^B	mU/l	15.15	15.15
Γ_{BU}^B	mg/min	2.3768	70
Γ_{RBCU}^B	mg/min	0.3395	10
Γ_{SU}^B	mg/min	0.6791	20
Γ_{HGP}^B	mg/min	5.263	155
Γ_{HGU}^B	mg/min	0.6791	20
Γ_{PGU}^B	mg/min	1.1884	35
K	min^{-1}	0.035	0.00794
α	min^{-1}	0.05	0.0482
β	min^{-1}	0.6	0.931
M_1	min^{-1}	0.27	0.00747
M_2	min^{-1}	0.9	0.0958
γ	U/min	0.0108	0.575
Q_0	U	0.0288	6.33
δ	Dimensionless	2	1.11

regional distribution (%) in humans and rats. From a total cardiac output of $85.05 ml/min$ [Delp et al., 1998], flows distribution to the organs were determined following the methodology developed in Sorensen [1985]. The data corresponding to an adult man of $70 kg$ was taken into account, the total weight of the organs was $34.96 kg$. In this work, an adult rat of $0.277 kg$ body weight was modeled and the corresponding total organs weight was considered of about $0.138 kg$ and the adopted total volume of blood was $20.7 ml$ [Hall et al., 2012].The resulting vascular parameter values as incorporated into the glucose model are included in Table 1. The total distribution volume for glucagon can be assumed equal to of insulin [Sorensen, 1985]. Thus, summing the vascular and interstitial fluid volumes of the insulin model gives a glucagon distribution volume of $V_N = 0.0473$ l. In compliance with Oshima et al. [1988], the metabolic glucagon clearance rate is $F_{PNC} = 0.0180$ l/min. All the metabolic rates are based on the basal value of the hepatic glucose production rate which is $\Gamma_{HGP}^B = 5.263$ mg/min [Rossetti et al., 1993]. Then, this source is distributed proportionally to the sinks analogously to the human model of Sorensen [1985] as can be seen in Table 1. It was assumed that the fractional clearance of insulin by the liver, peripheral tissues and kidney remained the same as for humans (F_{PC}, F_{KC} and F_{LC}). The same assumption was done for the diffusion time constants of glucose, glucagon and insulin for mass transfer (T_B, T_P^G, T_P^I, T_I and T_R^I). For the initialization of the model, the basal conditions were used the same as well: G_L^B, G_{PI}^B, G_H^B, I_L^B, I_{PI}^B and I_H^B.

3. Results and Conclusions

Figure 1 shows the evolution of all the states of the healthy subject simulator [Sorensen, 1985] after a 50 grams carbohydrate meal. The first subplot from the grid corresponds to

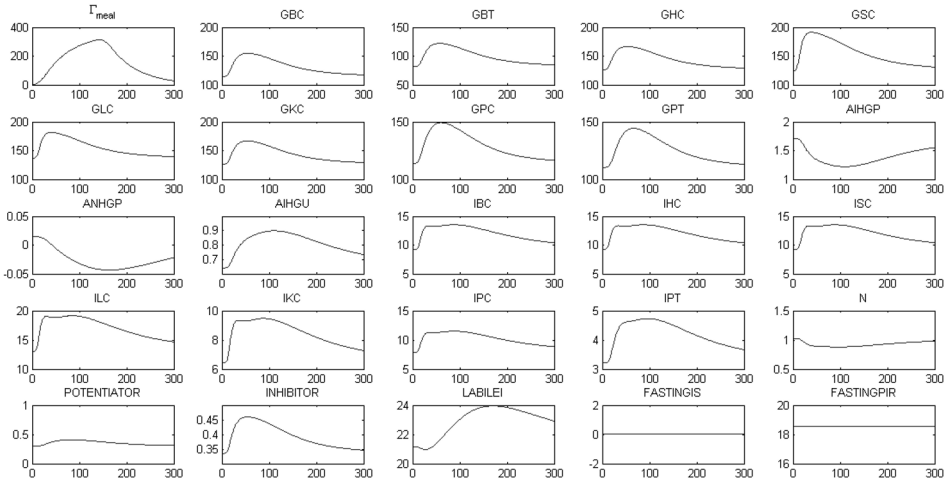

Figure 1: Simulation of Sorensen's normal human after a 50 gr meal.

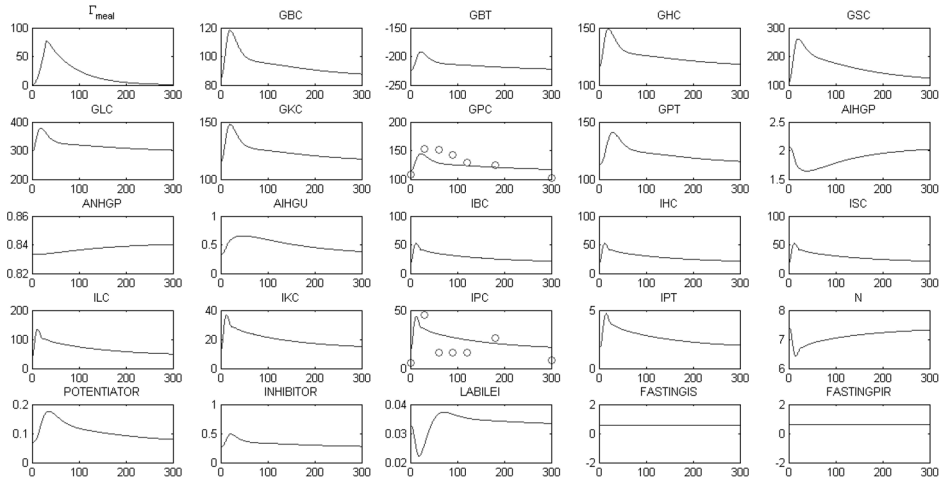

Figure 2: Simulation of Sorensen's model scaled to normal rat after a 2.2 gr meal.

the rate of glucose appearance of Dalla Man et al. [2006], equation 5. From subplot number 2 to 15, the subplots represents the states of the model of Sorensen, from equation 11 to 26 and the last five are from the insulin secretion model of Landahl and Grodsky [1982]. The comparison is made with a corresponding intake of 2.2 grams for the rat model in Figure 2. As can be seen, the evolution of the rat model is qualitatively and quantitatively similar to that of humans as expected. The points in red are real measurements from the laboratory of capillary blood glucose and insulin. The techniques employed are described in detail in the Appendix 5. Even though, further research should be done to improve the response. The next logical step is to contrast the results with more data from the laboratory. This is part of the future work of our team which has been carrying out experiments with the endocrine system of rats since 4 years ago. Additionally, many of the hypothesis

assumed should be thoroughly tested and confirmed. A model with such characteristics could be really useful to predict human responses when experimenting with rats. Consequently, safety procedures can be conducted. It could serve as an observer also, as the model sees the evolution of concentrations in every organ of the full body, all of which are very difficult to measure *in vivo*.

The authors want to acknowledge the invaluable help of Eng. Patricio Colmegna from ITBA, Argentine and the financial support from CONICET, Argentine.

4. Appendix 1: the mathematical model

The rate of glucose appearance (Γ_{meal}) of Dalla Man et al. [2006] is modeled by a three-compartment model:

$$\dot{Q_{sto1}}(t) = -k_{gri}Q_{sto1}(t) + d(t) \tag{1}$$

$$\dot{Q_{sto2}}(t) = -k_{empt}(t, Q_{sto}(t))Q_{sto2}(t) + k_{gri}Q_{sto1}(t) \tag{2}$$

$$\dot{Q_{gut}}(t) = -k_{abs} + k_{empt}(t, Q_{sto}(t))Q_{sto2}(t) \tag{3}$$

$$Q_{sto}(t) = Q_{sto1}(t) + Q_{sto2}(t) \tag{4}$$

$$\Gamma_{meal} = fk_{abs}Q_{gut}(t)/BW \tag{5}$$

where Q_{sto} (mg) is the amount of glucose in the stomach (solid, Q_{sto1} , and liquid phase, Q_{sto2}), Q_{gut} (mg) is the glucose mass in the intestine, k_{gri} is the rate of grinding, k_{abs} is the rate constant of intestinal absorption, f is the fraction of intestinal absorption which actually appears in plasma, $d(t)$ (mg/min) is the amount of ingested glucose, BW (kg) is the body weight, Γ_{meal} (mg/kg/min) is the glucose rate of appearance in plasma and k_{empt} is the rate constant of gastric emptying which is a time-varying nonlinear function of Q_{sto}:

$$k_{empt}(t, Q_{sto}(t)) = k_{max} + \frac{k_{max} - k_{min}}{2}[A(t)]; \tag{6}$$

where:

$$A(t) = \tanh[\alpha(Q_{sto}(t) - bD(t))] - \tanh[\beta(Q_{sto}(t) - dD(t))] \tag{7}$$

$$\alpha = \frac{5}{2D(t)(1-b)} \tag{8}$$

$$\beta = \frac{5}{2D(t)d} \tag{9}$$

$$D(t) = \int_{t_i}^{t_f}(t)dt \tag{10}$$

with t_i and t_f, respectively, start time and end time of the last meal, b, d, k_{max} and k_{min} model parameters.
From Sorensen [1985], the equations for glucose dynamics are:

$$\dot{G_B^C} = (G_H^C - G_B^C)\frac{q_B}{v_B^C} - (G_B^C - G_B^T)\frac{v_B^T}{T_B v_B^C} \tag{11}$$

$$\dot{G_B^T} = (G_B^C - G_B^T)\frac{1}{T_B} - \frac{\Gamma_{BU}}{v_B^T} \tag{12}$$

$$\dot{G_H^C} = (G_B^C q_B + G_L^C q_L + G_K^C q_K + G_P^C q_P - G_H^C q_H - \Gamma_{RBCU})\frac{1}{v_H^C} \tag{13}$$

$$\dot{G_S^C} = (G_H^C - G_S^C)\frac{q_S}{v_S^C} + \frac{\Gamma_{meal}}{v_S^C} - \frac{\Gamma_{SU}}{v_S^C} \tag{14}$$

$$\dot{G_L^C} = (G_H^C q_A + G_S^C q_S - G_L^C q_L)\frac{1}{v_L^C} + \frac{\Gamma_{HGP}}{v_L^C} - \frac{\Gamma_{HGU}}{v_L^C} \tag{15}$$

$$\dot{G_K^C} = (G_H^C - G_K^C)\frac{q_K}{v_K^C} - \frac{\Gamma_{KE}}{v_K^C} \tag{16}$$

$$\dot{G_P^C} = (G_H^C - G_P^C)\frac{q_P}{v_P^C} + (G_P^T - G_P^C)\frac{\Gamma_{v_P^T}}{T_P^G v_P^C} \tag{17}$$

$$\dot{G_P^T} = (G_P^C - G_P^T)\frac{1}{T_P^G} - \frac{\Gamma_{PGU}}{v_P^T} \tag{18}$$

Equations for insulin dynamics:

$$\dot{I_B^C} = (I_H^C - I_B^C)\frac{Q_B}{V_B^C} \tag{19}$$

$$\dot{I_H^C} = (I_B^C Q_B + I_L^C Q_L + I_K^C Q_K + I_P^C Q_P - I_H^C Q_H + \Gamma_{IVI})\frac{1}{V_H^C} \tag{20}$$

$$\dot{I_S^C} = (I_H^C - I_S^C)\frac{Q_S}{V_S^C} \tag{21}$$

$$\dot{I_L^C} = (I_H^C Q_A + I_S^C Q_S - I_L^C Q_L)\frac{1}{V_L^C} + \frac{\Gamma_{PIR}}{V_L^C} - \frac{\Gamma_{LC}}{V_L^C} \tag{22}$$

$$\dot{I_K^C} = (I_H^C - I_K^C)\frac{Q_K}{V_K^C} - \frac{\Gamma_{KC}}{V_K^C} \tag{23}$$

$$\dot{I_P^C} = (I_H^C - I_P^C)\frac{Q_P}{V_P^C} + (I_P^T - I_P^C)\frac{V_P^T}{T_P^I V_P^C} \tag{24}$$

$$\dot{I_P^T} = (I_P^C - I_P^T)\frac{1}{T_P^I} + \frac{\Gamma_{SIA}}{V_P^T} - \frac{\Gamma_{PC}}{V_P^T} \tag{25}$$

and the remaining 4 equations of Sorensen's model are:

$$\dot{N} = (\Gamma_{PNR} - N)\frac{F_{PNC}}{V_N} \tag{26}$$

$$\dot{A_{IHGP}} = \frac{1}{T_I}\left\{1.2088 - 1.138\tanh\left[1.669\left(\frac{I_L^C}{I_L^B} - 0.8885\right)\right] - A_{IHGP}\right\} \tag{27}$$

$$\dot{A_{NHGP}} = \frac{1}{T_R}\left[\frac{2.7\tanh(0.388N) - 1}{2} - A_{NHGP}\right] \tag{28}$$

$$\dot{A_{IHGU}} = \frac{1}{T_I}\left[2\tanh\left(0.549\frac{I_L^C}{I_L^B}\right) - A_{IHGU}\right] \tag{29}$$

Metabolic rates causing addition or removal of mass were assigned mathematical equations of the general form:

$$\Gamma_e = E_{\Gamma_e}\{A_{\Gamma_e} - B_{\Gamma_e}\tanh[C_{\Gamma_e}(x_i + D_{\Gamma_e})]\} \tag{30}$$

The subscript i in Eq. 30 is the state vector element involved in the metabolic effect, and subscript e denotes specific effects within the model: the effect of glucose on hepatic glucose production, the effect of glucose on hepatic glucose uptake, and the effect of insulin on peripheral glucose uptake. These equations were used with minor modifications. The pancreatic insulin release model (Γ_{PIR}) used in Sorensen's model was developed by Landahl and Grodsky [1982] and for the lack of space is also omitted.

5. Appendix 2: experimental procedures

5.1. 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 12h/12h and temperature of

23 ± 1 degree centigrade. Blood samples were obtained from the vein of the tail in heparinized tubes; they were centrifuged and plasma was saved at 20 degrees centigrade to measure glucose and insulin concentrations. All experiments were performed in accordance with the international ethical guidelines of animal care [Nat, 1985, Olfert et al., 1993]. The protocol was approved by the Ethics Committee, School of Medicine, Rosario National University.

5.2. Glucose oral administration

Animals with 8 h of fast received glucose (0,6g/100g 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 minutes).

5.3. Glucose Measurement

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

5.4. 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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