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A non-linear mixed-effects model to describe the effect of acarbose intake on postprandial glycaemia in a single rat

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ABSTRACT. A non-linear mixed-effects model is proposed to assess the impact of acarbose over time on postprandial glycaemia in a single rat. The model is based on two compartments, one representing the entry of glucose in the blood and the other its exit. The rat was submitted to two treatments: ingestion of starch and ingestion of starch plus acarbose. The model showed great suitability, with inferences on the behavior of glucose levels in response to treatments and supplying a richer description than just the area under the curve. The marginal curves for the two treatments are similar during the first moments; however, after reaching the peak of glucose concentration, they progressively became separate due to acarbose treatment and reached the initial levels more quickly. The proposed model, albeit with a single sample unit, showed similar results to those with larger samples; in other words, acarbose significantly attenuates glycaemia after ingestion of starch.

Keywords: Diabetes mellitus, glucose, small sample, two-compartment model.

Um modelo não linear de efeitos mistos para descrever o efeito da ingestão de acarbose na glicemia pós-prandial em um único rato

RESUMO. Neste estudo, foi proposto um modelo não linear de efeitos mistos para verificar o impacto da acarbose ao longo do tempo na glicemia pós-prandial de um único rato. Adotou-se um modelo de dois compartimentos: um representando a entrada de glicose no sangue e outro, a saída. O rato foi submetido a dois tratamentos: ingestão de amido e de amido com adição de acarbose. O modelo proposto apresentou um ótimo ajuste, permitindo fazer inferências do comportamento da glicose para os tratamentos e fornecendo uma descrição muito mais rica do que simplesmente a área sob a curva. As curvas marginais para os dois tratamentos foram semelhantes nos primeiros tempos observados, porém, após o pico de concentração de glicose, elas se distanciaram progressivamente com o tratamento da acarbose atingindo os níveis iniciais mais rapidamente. O modelo adotado, com uma única unidade amostral, mostrou resultados similares a outros estudos com maior número de unidades amostrais, isto é, a acarbose pode atenuar consideravelmente a glicemia após ingestão de amido.

Palavras-chave: Diabetes mellitus, glicose, modelos de dois compartimentos, pequenas amostras.

Introduction

The metabolic disease diabetes mellitus is among the ten mortality causes in populations worldwide. The high cost of the disease demands that public and private health systems investigate new treatments, intervention programs (Ejtahed et al., 2012; König, Kookhan, Schaffner, Deibert, & Berg, 2014; Shen, Obin, & Zhao., 2013; Costa & Longo, 2014) and drugs capable to improve the patients' quality of life. In living beings, the α-amylases are enzymes that catalyze the hydrolysis of polysaccharides, such as starch and glycogen, to yield glucose and maltose (Wang et al., 2008). However, some organic compounds, among them acarbose, may inhibit the activity of these enzymes (Geng & Bai, 2008; Ritz et al., 2012) and thus prevent or attenuate hyperglycemic peaks (Coniff, Shapiro, Seaton, & Bray, 1995; Pereira et al., 2011; Scheen, Magalhaes, Salvatore, & Lefebvre, 1994; Sybuia, Guilhermetti, Mangolim, Bazotte, & Matioli, 2014-2015; Wong & Jenkins, 2007). Acarbose has been widely studied for the treatment of Type 2 diabetes (Ritz et al., 2012; Rosak, Haupt, Walter, & Werner, 2002; Yee & Fong, 1996) as a therapeutic agent added to food or as a drug administered orally (Espín, García-Conesa, & Tomás-Barberán, 2007; Eng Kiat Loo & Huang, 2007).

Several researchers have been trying to understand better the behavior of the disease and the

effects of new treatments. However, besides economic factors, the policy of committees for ethics on studies involving animals and humans, which generally recommend the use of smaller numbers in samples should be taken into account. This fact makes it hard to obtain sufficient data to reach statistically robust results. Hence, it is important to propose methods that would accommodate inherent characteristics of research in health and biology.

Non-linear models have been proposed in the literature to obtain a better understanding of diabetes (Ajmera, Swat, Laibe, Le Novère, & Chelliah, 2013; Boutayeb & Chetouani, 2006; Briegel & Tresp, 2002; Caumo, Saccomani, Toffolo, Sparacino, & Cobelli, 1999; Hovorka et al., 2004; Wu, 2005). Nevertheless, many studies with longitudinal data on glycemia only calculate the area under the curve (AUC) to compare treatments (Sybuia et al., 2014-2015; Tai, 1994). In current research, a non-linear mixed-effects model is investigated to determine the impact of acarbose on postprandial glycemia in a single rat after the ingestion of soluble starch. The analysis was carried out with R Statistical Software (R Core Team, 2015) using NLME package (Pinheiro, Bates, Debroy & Sarkar, 2007).

Material and methods

Current assay was performed with a single adult male Wistar rat. The experimental protocol was approved by the Committee for Ethics of the State University of Maringá, Maringá, Paraná State, Brazil, following international law on the protection of animals. The rat was maintained under constant temperature (22°C ± 1°C) with automatically controlled photoperiod (12h light/12h dark).

The rat received two treatments, or rather, one was given by gavage at 1 g kg^{-1} of soluble starch and the other contained the same quantity of starch plus 10 mg kg^{-1} acarbose. Soluble starch was obtained from Merck (Darmstadt, Germany) and acarbose (Glucobay®) from Bayer (São Paulo, Brazil). The glucose sensor device was obtained from Medtronic (São Paulo, Brazil).

One day prior to the experiment, the animal was made to fast for 12 h (8:00 p.m.–8:00 a.m.) to discard any interference of intestinal absorption of glucose. At 8:00 a.m., the rat received 1 g kg^{-1} soluble starch by gavage. During 65 minutes after this application, the glucose concentrations in the rat's blood were recorded at every five minutes (until 09:05 a.m.). Three hours after the administration of starch (at 11:00 a.m.), the rat received 10 mg kg^{-1} of acarbose by gavage. Immediately the rat received 1 g kg^{-1} of soluble starch by gavage and glucose levels were recorded every 5 minutes until 12:05 p.m. At 13:30 p.m., the animal was given free access to water and food, and at 20:00 p.m., the animal fasted for 12 h once more. The procedure was repeated for three consecutive days.

The sequence adopted for treatments guaranteed that no residual acarbose influenced the results. Moreover, although the rat is a nocturnal animal, the long period of fasting made it eat in the morning (no significant weight loss was registered during the three days of experiment).

Glucose was measured by a real-time continuous glucose monitoring system (RT-CGMS) technique (Woderer et al., 2007; Carrara et al., 2012; Tavoni et al., 2013). The RT-CGMS is a portable device that requires insertion of a glucose sensor in the animal's subcutaneous tissue. RT-CGMS evaluates glucose levels every 10 sec and the results obtained every 5 as the average sum of 30 glucose concentration rates were sent by radio to a computer for analysis.

Figure 1 shows data on postprandial glucose concentration over time, grouped by day and by treatment. One may observe that all profiles have a clear pattern, with the glucose level in the blood rising quickly at the start followed by a gradual decline. Although as a rule baseline and stabilization of glucose concentration depends on the individual condition, the pattern of glucose levels in current assay is related to the moment when the rat received the treatments.

In pharmacokinetic models, the human body is usually represented as a system of compartments, in which the drug is transferred according to a first-order or zero-order kinetic equation (Gibaldi & Perrier, 1982). The drug's concentration in the different compartments and over time is determined by a system of differential equations whose solution may be expressed as a linear combination of exponential functions. Similarly, the model used in this study represents the changes in postprandial glucose over time as a process with two compartments: the first representing the absorption of glucose in the blood and the second its elimination.

The ordinary differential equation (ODE) for each compartment represents the variation of postprandial glucose as being proportional to the time since administration and to the amount of glucose at that instant. For example, in the absorption period, the glucose concentration in the blood monotonically increases with time, or rather, its rate of variation grows from zero point (when no absorption has yet occurred) and then declines until it returns to zero again (when the absorption period ends).

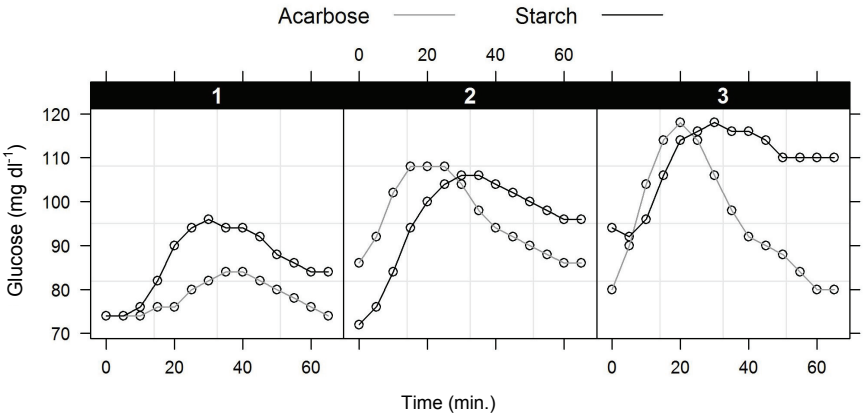

Figure 1. Glycemic curves after oral administration of starch and soluble starch plus acarbose after three days of experiment in a single rat fasted for 12 hours. Glucose levels were recorded every five minutes with the use of RT-CGMS.

Therefore, ODE may be written as

$$\frac{dG_1}{dt} = k_1 t G_1 \quad (1)$$

where:
G_1 is the glucose absorption function; t is time; k_1 is the constant of proportionality. The solution of this equation leads to

$$G_1(t) = c_1 \exp(k_1 t^2) \quad (2)$$

where:
c_1 and k_1 are constants that represent the intercept and the shape of G_1 respectively. The elimination process starts immediately after ingestion of the starch and lasts until the glucose level reaches normal rates. Analogously to the absorption period, the glucose elimination function is given by

$$G_2(t) = c_2 \exp(k_2 t^2). \quad (3)$$

The constants k_1 and k_2 correspond respectively to absorption and elimination rates.

Unlike pharmacokinetic models, the final model must also have an intercept, since the human body tries to maintain the glucose level fluctuating around a constant rate. Thus, denoting the glucose concentration for profile i at time t_j, with $i = 1, \ldots, 6$ and $j = 1, \ldots, 14$, by G_{ij}, the final model is a linear combination of Equations 2 and 3

$$G_{ij} = \phi_{0i} + \phi_{1i} \exp(\phi_{2i} t_j^2) + \phi_{3i} \exp(\phi_{4i} t_j^2) + \varepsilon_{ij}, \quad \phi_{2i} < 0, \ \phi_{4i} < 0 \quad (4)$$

in which

$$\boldsymbol{\phi}_i = \begin{bmatrix} \phi_{0i} \\ \phi_{1i} \\ \phi_{2i} \\ \phi_{3i} \\ \phi_{4i} \end{bmatrix} = \begin{bmatrix} \beta_0 + \gamma_0 x_i \\ \beta_1 + \gamma_1 x_i \\ \beta_2 + \gamma_2 x_i \\ \beta_3 + \gamma_3 x_i \\ \beta_4 + \gamma_4 x_i \end{bmatrix} + \begin{bmatrix} b_{0i} \\ b_{1i} \\ b_{2i} \\ b_{3i} \\ b_{4i} \end{bmatrix} = \boldsymbol{\beta} + \boldsymbol{\gamma} x_i + \boldsymbol{b}_i, \quad (5)$$

with the fixed effects β representing the mean values of the parameters Φ_i, and the random effects $\boldsymbol{b}_i \sim N(0, \boldsymbol{\Psi})$ representing the deviations of β, considered to be independent among the profiles. The treatment effect is specified in the model by the parameters γ, with $x_i = 0$ if the treatment is starch alone and $x_i = 1$ if the treatment is starch with acarbose. The errors $\varepsilon_{ij} \sim N(0, \sigma^2)$ are considered to be independent of the random effects and for the different i and j rates.

Since the parameters Φ_2 and Φ_4 must be negative to make biological sense, we re-parameterized the model in terms of $\phi_2' = \log(-\phi_2)$ and $\phi_4' = \log(-\phi_4)$ (Pinheiro & Bates, 2000). Hence, the model does not have any restrictions with regard to the parameters.

Results and discussion

Since the number of profiles was very near the number of random effects in the model, we were unable to use a positive defined matrix with all the possible covariances (Pinheiro & Bates, 2000). Therefore, we initially used a diagonal matrix with all the parameters to specify the structure of the covariances of the random effects, Ψ.

Analyzing the estimates of the random effects with respect to the treatments, we observed a possible systematic pattern of the parameter Φ_0. After fitting the complete model and various reduced models, we chose the model with only γ_0, γ_3 and γ_4 by calculating AIC (Akaike, 1974) and BIC (Schwarz, 1978) rates and applying the likelihood ratio test. Employing this model, the estimated standard deviation of the random effect for Φ_1 was nearly zero (the parameter Φ_0 accommodated all the variability of the intercept of the first exponential equation). After testing some structures for the random effects, we chose the diagonal matrix without effect for Φ_1. The estimated rates and 95% confidence intervals for the fixed effects and for the standard deviations of the random effects are reported in Table 1. Recall that $\beta_2 = -\exp(\beta_2')$ and $\beta_4 = -\exp(\beta_4')$.

The first two diagnostic graphs in Figure 2 (of the standardized residuals versus the estimated values and the observed values versus the estimated values) do not indicate large deviations from the proposed nonlinear model.

Figure 3 shows a quantile-quantile (Q-Q) graph for the assumption of normal distribution of the residuals. The linearity of the points suggests no serious violation of this assumption.

Another evaluation of the model's adequacy is provided by comparing the individual profiles (observed rates) and the conditional profiles (obtained when the estimates of the random effects are used) and marginal profiles (corresponding to the fixed effects), as presented in Figure 4. Note that the conditional predictions are near the observed concentrations, indicating that the model provides a good representation of the data.

Table 1. Estimates, lower and upper bounds (LB and UB) for the model's parameters.

	LB	Estimate	UB		LB	Estimate	UB
β_0	84.6104	94.7356	104.8608	σ_{b_0}	4.7892	8.6508	15.6258
β_1	-65.5407	-56.9421	-48.3436				
β_2'	-6.4698	-5.9670	-5.4642	σ_{b_2}	0.3324	0.6027	1.0929
β_3	31.7150	40.9461	50.1772	σ_{b_3}	1.5476	3.2901	6.9946
β_4'	-7.4754	-7.1238	-6.7721	σ_{b_4}	0.1115	0.2451	0.5390
γ_0	-30.3190	-15.9917	-1.6644	σ	1.1269	1.3660	1.6558
γ_3	11.4104	17.6471	23.8838				
γ_4	-0.3817	0.0645	0.5108				

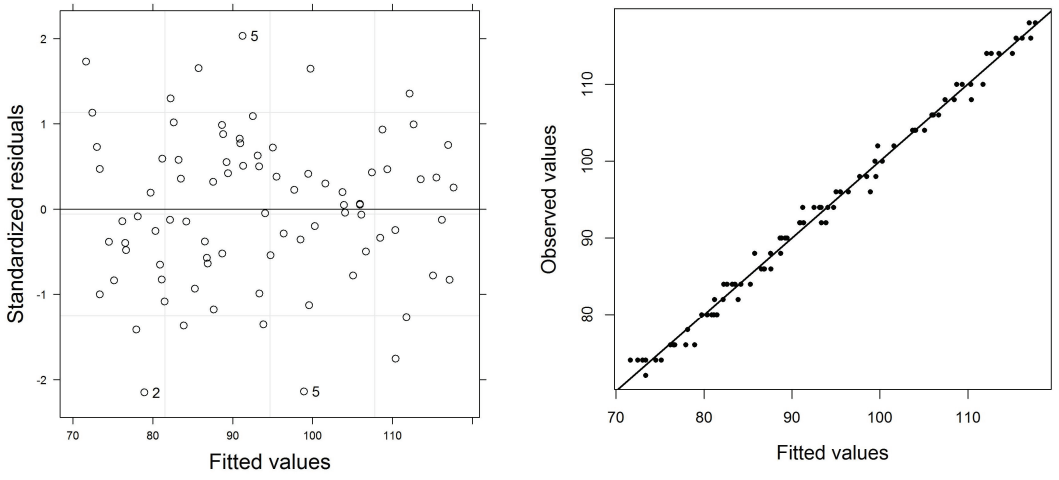

Figure 2. Diagnostic graphs. The left graph plots the standardized residuals versus the fitted rates, while the right panel shows the observed rates versus the fitted ones (the straight line represents a perfect fit).

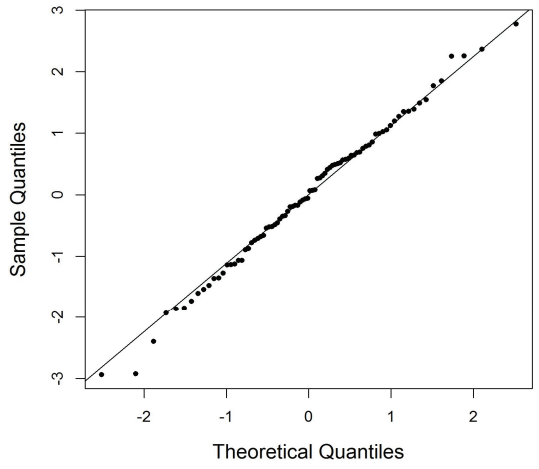

Figure 3. Normal Q-Q plot for the residuals (the linearity of the points indicate a good fit to the normal distribution).

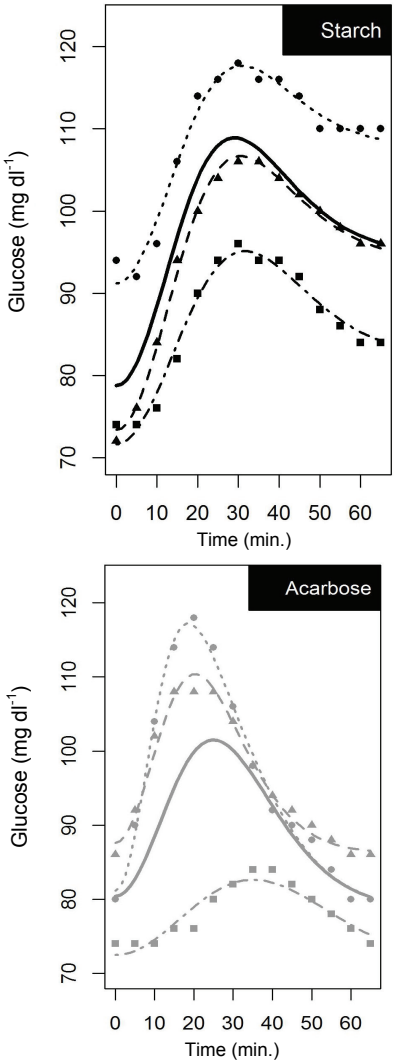

Figure 4. Scatter plot for the observed glucose levels after oral administration of starch (left panel) and soluble starch plus acarbose (right panel), together with the conditional (thin lines) and marginal (thick lines) profiles. Each kind of point or line represents a different profile.

The area under the curve (AUC) is a common measure to compare glucose curves. To calculate AUC, we integrated the marginal models estimated for the two treatments in the interval between 0 and 65 min, and subtracted from this rate the area below 70 mg dl^{-1} (none of the experimental data was below this cutoff). AUC for the treatment with starch alone was 1.877 mg dl^{-1} min. while that for the treatment plus acarbose was 1.330 mg dl^{-1} min., approximately 29% smaller.

Another comparative method is to calculate the maximum estimated glucose concentration and report the time the rate is reached; also to find the levels and times of the first and second inflection points, which represent the maximum absorption and elimination, respectively. For the starch curve, the maximum concentration was 108.9 mg dl^{-1} and the time was 29 min; in the case of the acarbose curve, the concentration was lower, approximately 101.5 mg dl^{-1}, at a shorter time, at 25 min. As expected, the maximum absorption of acarbose and starch occurred at similar times (around 12 minutes), but the maximum elimination of starch and acarbose occurred at 41 and 38 min. respectively.

Figure 5 presents the fitted marginal model G, the two exponential functions that compose it (G_1 and G_2) and its rate of variation (G') for the two treatments. The behavior of the curve that represents glucose absorption by the blood (G_1) is equal for the two treatments, increasing from negative values and asymptotically approaching zero. However, the elimination process is substantially different. Thus, the variation rate of glucose concentration changes over the entire period between the treatments, observed in the area under the curve of G'. The positive area is greater for starch, implying a higher glucose concentration in the blood, while the negative area is greater for acarbose, implying that the glucose concentration declined more quickly.

The process of absorption and elimination of glucose in the blood is dynamic. The human body maintains the homeostasis of glucose levels in the blood using insulin and glucagon. Even during long fasting periods, the glucose levels do not decline drastically and glucose absorption and elimination rates in the blood are kept relatively stable. However, after eating food rich in carbohydrates, the alteration of the absorption and elimination rates raises the level of glucose in the blood. When the absorption process ends, the elimination persists longer until the glucose concentration reaches its reference value again. In this study, we estimated the reference values at 94.74 and 78.74 mg dl^{-1} for the treatment with starch and acarbose, respectively. The 65-minute duration was only sufficient for the glucose level to return to rates close to the initial ones in the case of acarbose.

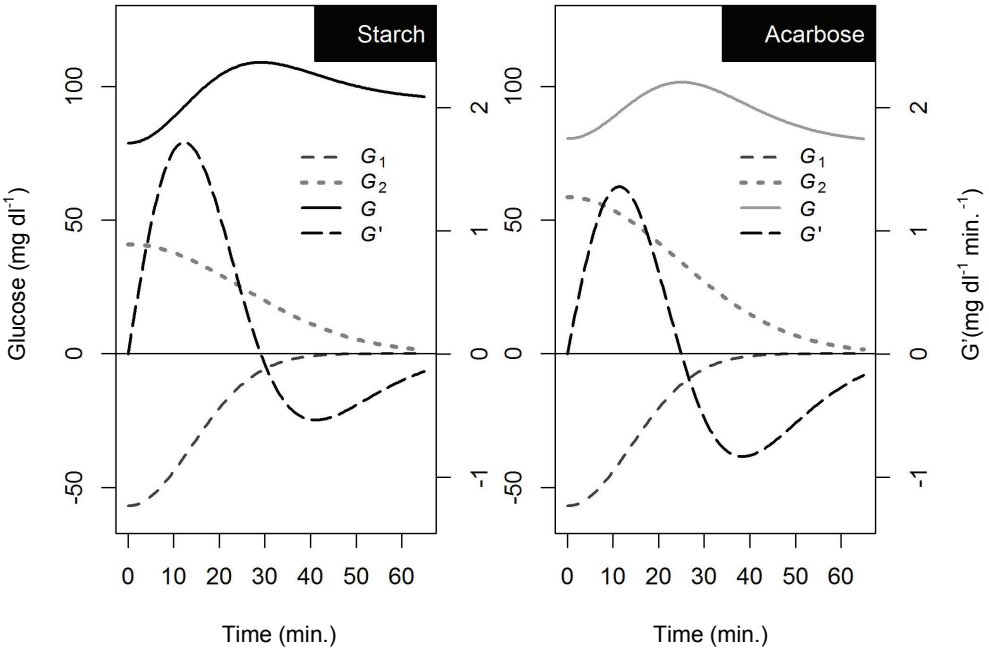

Figure 5. Fitted marginal model (G), the two exponential functions that compose it (G_1 and G_2) and its rate of variation (G') for the treatment with starch (left panel) and the treatment with soluble starch plus acarbose (right panel).

Conclusion

The use of animals for scientific purposes has many advantages. However, due to internal pressures on the scientific community to optimize resources and to external pressures from animal protection groups, the number of animals for research should be minimized. Hence, the need to work with few samples in health and biological sciences is growing, prompting statisticians to improve their methods. In current study, with only one experimental unit (a single rat), it was possible to obtain results similar to those of other studies which reported the effect of acarbose on glycaemia carried out with larger samples (Coniff et al., 1995; Pereira et al., 2011; Ritz et al., 2012; Rosak et al., 2002; Scheen et al., 1994; Sybuia et al., 2014-2015; Wong & Jenkins, 2007; Yee & Fong, 1996).

Further, the modified two-compartment model could be applied to a variety of metabolic processes in which the same pattern is observed. The model seems ideal to describe phenomena that may be represented by the entry and the exit of a substance from a homeostatic system (a system with dynamic equilibrium).

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