Effect of acarbose intake on postpandrial glycemia in a single rat - a nonlinear mixed effects model

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Abstract

The objective of this study was to determine the impact of acarbose on the postprandial glycemia of a single rat after ingestion of soluble starch. We adopted a nonlinear mixed effects model with two compartments, one representing the entry of glucose in the blood and the other the exit, to ascertain the data on postprandial glucose concentration over time, of a single rat submitted to two treatments, ingestion of starch and ingestion of starch plus acarbose. Analysis of the proposed model showed good fit, allowing inferences about the behavior of glucose levels in response to treatments and supplying a richer description than just the area under the curve (AUC). For a single sample unit, the proposed model showed that acarbose can attenuate glycemia after ingestion of starch, with similar results to those found by other studies.

Keywords: diabetes mellitus; glucose; small sample; two-compartment model.

1. Introduction

The metabolic disease diabetes mellitus is highly prevalent in the global population, among the 10 leading causes of death. The high cost the disease imposes on public and private health systems is a strong reason to investigate new treatments, intervention programs [6, 11, 22] and drugs able to improve patients' quality of life. In living beings, the α-amylases are enzymes that catalyze the hydrolysis of polysaccharides like starch and glycogen, yielding glucose and maltose [25]. However, some organic compounds, among them acarbose, can inhibit the action of these enzymes [8, 19], preventing or attenuating hyperglycemic peaks [5, 14, 15, 21, 23, 26]. Acarbose has been widely studied for treatment of type 2 diabetes [13, 19, 20], as a therapeutic agent that can be added to food or as a drug administered orally [7, 12].

Various researchers have been trying to gain a better understanding of the behavior of the disease and the effects of new treatments. However, besides economic factors, consideration must be given to the policies of ethics committees on studies involving animals and humans, which generally urge the use of small numbers of sample units. This makes it hard to obtain sufficient data to reach statistically robust results. Hence, it is important to formulate methods that can accommodate this inherent characteristic of research in the areas of health and biology.

Nonlinear models have been proposed in the literature to obtain a better understanding of diabetes [1, 2, 3, 4, 10, 27]. Nevertheless, many studies with longitudinal data on glycemia only calculate the area under the curve (AUC) to compare treatments [23, 24]. Here we report the use of a nonlinear mixed model to determine the impact of acarbose on postprandial glycemia in a single rat after ingestion of soluble starch. The analysis was carried out with the R statistical software [18] with use of the **nlme** package [17].

2. Procedures and measurements

This study was performed with a single adult male Wistar rat, with weight of 275 g. During the study period, the rat remained under constant temperature (23 ^{o}C) with an automatically controlled photoperiod. The rat received two treatments on the same day: administration by gavage of 100 mg/kg of body mass of starch in the morning, and in the afternoon administration of the same quantity of starch plus 10 mg/kg of

acarbose. During 65 minutes after each treatment, the glucose concentrations in the rat's blood were recorded every five minutes. This procedure was repeated for three consecutive days. The glucose was measured by a portable device that requires insertion of a glucose sensor in the animal's subcutaneous tissue. The glucose concentration values were sent by radio to a computer for analysis.

3. Model formulation

The data on postprandial glucose concentration over time, grouped by day and by treatment, are presented in Figure 1. It can be seen that all the profiles have a clear pattern, with the glucose level in the blood rising quickly at the start, followed by gradual decline. In general, the baseline and stabilization of the glucose concentration depend on the individual condition. However, in this case the pattern of the glucose levels is related to the moment when the rat received the treatments.

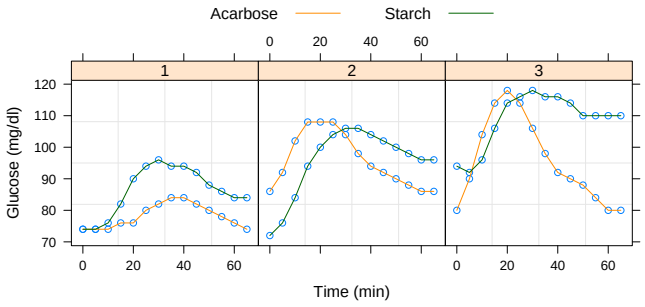

Figure 1: Glycemic curve for the starch and acarbose treatments during three days, at 14 different moments.

In pharmacokinetic models it is common to represent the human body as a system of compartments, in which the drug is transferred according to a first-order or zero-order kinetic equation [9]. The drug's concentration over time in the different compartments is determined by a system of differential equations whose solution can 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 the amount of glucose at that instant. For example, in the absorption period, the glucose concentration in the blood is monotonically increasing with time, but its rate of variation grows from the zero point (when no absorption has yet occurred) and then declines until it returns to zero again (when the absorption period ends). Therefore, the ODE can be written as

$$\frac{dG_1}{dt} = k_1 t G_1 \,, \tag{1}$$

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

$$G_1(t) = c_1 \exp(k_1 t^2) \,, \tag{2}$$

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 values. Analogously to the absorption period, the glucose elimination function is given by

$$G_2(t) = c_2 \exp(k_2 t^2) \,. \tag{3}$$

The constants k_1 and k_2 correspond to the absorption rate and elimination rate, respectively.

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 value. 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:

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

in which

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

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

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 $\phi_4' = \log(-\phi_4)$ [16]. Hence, the model does not have any restriction regarding the parameters.

4. Results and Discussion

Since the number of profiles is very near the number of random effects in the model, we were unable to use a positive defined matrix containing all the possible covariances [16]. Therefore, we initially used a diagonal matrix with all the parameters to specify the structure of the covariances of the random effects, $\boldsymbol{\Psi}$.

Analyzing the estimates of the random effects with respect to the treatments, we observed a possible systematic pattern of the parameter $\boldsymbol{\phi}_0$. After fitting the complete model and various reduced models, we chose the model with only γ_0, γ_3 and γ_4 by calculating the AIC and BIC values and applying the likelihood ratio test. With 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 values 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 $\beta_4 = -\exp(\beta_4')$.

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				

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 of the proposed nonlinear model. The last graph in the figure is a quantile-quantile 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 values) and the conditional profiles (obtained using the estimates of the random effects) and marginal profiles (corresponding to the fixed effects), as presented in Figure 3. Note that the conditional predictions are near the observed concentrations, indicating that the model provides a good representation of the data.

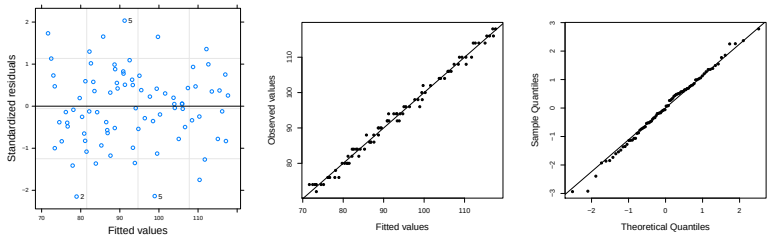

Figure 2: Diagnostic graphs.

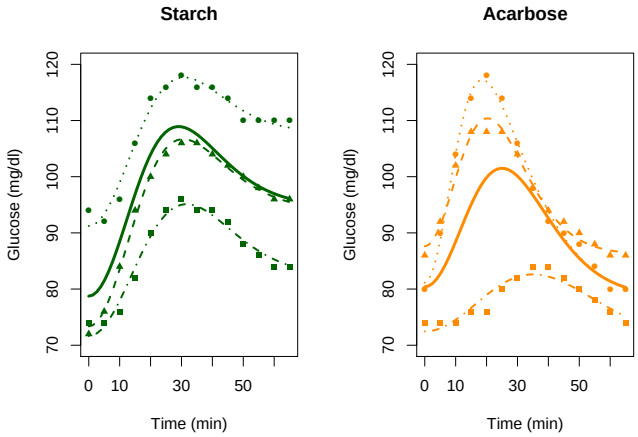

Figure 3: Model predictions at the individual and population levels, overlaid on observed data.

A common measure used to compare glucose curves is the area under the curve (AUC). To calculate the AUC, we integrated the marginal models estimated for the two treatments in the interval from 0 to 65 min, and subtracted from this value the area below 70 mg/dl (none of the experimental data were below this cutoff). The AUC for the treatment with only starch was 1,877 mg/dl min while that for the treatment including acarbose was 1,330 mg/dl min, about 29% smaller.

Another comparison method is to calculate the maximum estimated glucose concentration and note the time when this value is attained, and 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 and the time was 29 min, while for the acarbose curve the concentration was lower, about 101.5 mg/dl, and this happened sooner, at 25 min. As expected, the maximum absorption for acarbose and for starch occurred at similar times (around 12 minutes), but the maximum elimination for starch happened at 41 min and for acarbose at 38 min.

Figure 4 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 changed over the entire period between the treatments. This can be observed from 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,

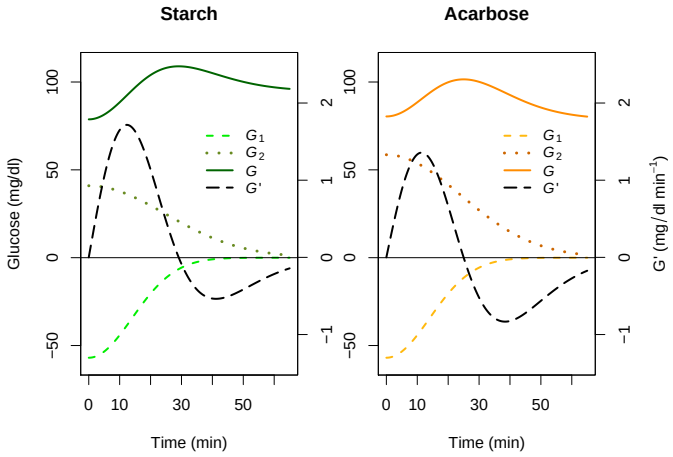

Figure 4: Fitted marginal model (G), the two exponential functions that compose it (G_1 and G_2) and its rate of variation (G') for the two treatments.

the glucose levels do not decline drastically and the 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. After 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 for the treatment with starch and acarbose, respectively. The time of 65 minutes was only sufficient for the glucose level to return to values near the initial ones in the case of acarbose.

5. Conclusions

The use of animals for scientific purposes has many advantages. However, due to internal pressures in the scientific community to optimize resources and external pressures from animal protection groups, there is a need to minimize the number of animals used for research. Hence, the need to work with small samples in the areas of health and biological sciences is growing, prompting statisticians to improve the existing methods. In this study, with only one experimental unit (a single rat), it was possible to obtain similar results to those of other studies reporting the effect of acarbose on glycemia, carried out with larger samples [5, 13, 14, 15, 19, 20, 21, 23, 26].

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