COMPARING LONGITUDINAL PROFILES BEFORE AND AFTER AN INTERVENTION

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▪ ABSTRACT: Occasionally, the behavior of a response variable monitored over time can be influenced by an intervention performed during the experimental period. With this perspective, this study proposes a simple methodology based on the fitting of two mixed effects models in longitudinal profiles, before and after an intervention, to verify significant differences. The notoriety of this methodology consists of using all repeated observations from the response variable regarding the intervention. This proposed method was motivated by two real datasets. Linear mixed models were fitted in the first dataset, which refers to the CD4 cells count in HIV-positive patients whom, over 30 consecutive days, received a glutamine based food supplement. For the second dataset, nonlinear mixed effects models were fitted for the body mass measurements of preterm newborns whose initial diet was based on breast milk and was subsequently replaced by a commercial food supplement. The proposed methodology was able to identify differences in the growth trend of the CD4 cells count after the observed patients took glutamine based supplementation. Moreover, it provided evidences suggesting the commercial food supplement as an alternative to a breast milk diet in preterm newborns by maintaining the body mass growth trend.

▪ KEYWORDS: Linear and nonlinear mixed model; longitudinal profile; preterm newborn; breast milk; HIV; glutamine.

1 Introduction

Studies which observes a variable or feature repeatedly over time are frequently in several areas (HOLBROOK *et al.*, 1995; KARLSSON *et al.*, 2000; VISSCHERS and SIEGRIST, 2013; YZERMANS *et al.*, 2005; HERAS *et al.*, 2002; RITCHIE *et al.*, 1998; SIVERTSEN *et al.*, 2015; CANNON *et al.*, 2015; TRAN *et al.*, 2015; DEL DUCA *et al.*, 2015). The purpose of these studies is to understand the variability between the observed variables from a single individual, in order to guide possible decisions. To exemplify, consider that a variable was observed several times over time in the same individual and, in a given moment, there were an intervention. The nature of this intervention can be nutritive, educational, social, surgical among others. For instance, one might be interested in comparing the criminality index, in several countries, before and after a sanction of the disarmament law. Therefore, the purpose is to verify the effectiveness of the intervention, hence, it is expected to identify possible changes in the before and after observations, with the intend to direct such effect to the intervention.

A known strategy to identify such differences, assuming the normality of the observed variable, would be to select the registered observations immediately before and after the intervention and perform a hypothesis test according to the paired t statistics (STUDENT, 1909). However, this possibility would result in the loss of information contained in the other observations which composes the subject response profile, disregarding the variability and correlation which describes them, represented by a covariance structure (FITZMAURICE *et al.*, 2012;

FITZMAURICE and RAVICHANDRAN, 2008; BROWN and PRESCOTT, 2014; FITZMAURICE *et al.*, 2008). To not consider these aspects could lead to different results and wrong decisions.

Regarding such haziness, it would be advantageous to employ all repeated observations of the variable to compare the profile of the same subject before and after an intervention, so that the inherent variability of the measures was weighted by the analysis. In this sense, what this study proposes meets this demand, presenting a simple methodology which appreciates all observations for the comparison of the same subject profile through an intervention. Such methodology is based on the mixed models approach, suggesting the addition of random effects into the parameters of the selected regression model to describe the observed variable, aiming the incorporation of a covariance structure presented on the data.

The application of the proposed methodology in given by two real datasets, the first one with 12 experimental units and the second with 8. Even though both could be treated as small sample cases, the repetitions on each experimental unit allows a greater quantity of observed values and increases the degrees of freedom of the proposed models. Furthermore, if the correlations are considered properly in the mixed model, the amount of experimental units may not limit the analysis (PEREIRA *et al.*, 2017). Consequently, the first dataset, fitted with a linear mixed effects model, refers to the CD4 cells count in HIV-positive patients whom, for 30 consecutive days, received a glutamine based food supplement. The second dataset, fitted with a nonlinear mixed effects model, concerns the body mass of preterm newborn admitted in a maternity, that initially received a breast milk diet and later a commercial food supplement. In both cases, the objective is to verify the effectiveness of the food interventions, i.e., if the diet changes resulted in the increase of CD4 cells in HIV-positive patients and an increase in the rate of change in body mass of preterm newborns, respectively. The analysis were developed with the software `R` (R CORE TEAM, 2018).

1.1 Methodology

The relevance of this proposed methodology refers to the usage of the subjects response profile in its entirety to compare it facing an intervention. The response profiles are composed by repeated observations over time establishing longitudinal profiles. The presence of the intervention splits the profiles into two moments, before and after the intervention. Thus, it is proposed mixed effects models for each profile moment, with the intention of modeling the variability of the repeated observations before and after the intervention. The fitting of these models allows the comparison of the respective rates of change of the responses over time, providing means to analyze the influence of the intervention in the longitudinal response profiles, as shown in Figure 1.

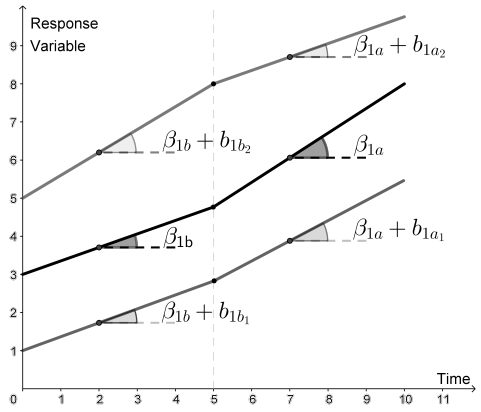

Figure 1 - The middle profile represents the mean profile, while the other two indicates individual longitudinal responses.

The mixed effects models establishes an important regression tool for correlated data and are defined by

$$y_i = g(\mathbf{X}_i, \mathbf{Z}_i, \boldsymbol{\beta}, \mathbf{b}_i) + \varepsilon_i, \quad i = 1, \cdots, n, \tag{1}$$

whereas $\mathbf{y}_i = (y_{i1}, \cdots, y_{ip_i})^T$ with dimension $(p_i \times 1)$ is the response profile of the i-th experimental unit, $\boldsymbol{\beta}$ is a vector with dimension $(t \times 1)$ of fixed effects, $\mathbf{X}_i = (x_{i1}, \cdots, x_{im})$ is a known specification matrix of fixed effects with dimension $(p_i \times t)$, $\mathbf{b}_i$ is a vector with dimension $(q \times 1)$ of random effects, $\mathbf{Z}_i$ is a known full-rank specification matrix of random effects, g is a function with continuous second order derivatives and $\boldsymbol{\varepsilon}_i$ is a vector of random errors with dimension $(p_i \times 1)$. Also, $\mathbf{b}_i \sim N_q(\mathbf{0}, \mathbf{G})$, and $\boldsymbol{\varepsilon}_i \sim N_{pi}(\mathbf{0}, \mathbf{R}_i)$ with $\mathbf{G}$ and $\mathbf{R}_i$ being symmetrical positive-define covariance matrix of order $(q \times q)$ and $(p_i \times p_i)$, respectively. Besides that, $\mathbf{b}_i$ and $\boldsymbol{\varepsilon}_i$ are independent random variables.

The presence of random variables in the model allows the fixed effects to vary randomly from one experimental unit to another, describing the individual behavior and incorporating the covariance structure inherent to repeated measurements data. For a better understanding, consider the particular case which g is a linear function of the fixed effects $\boldsymbol{\beta}$ and the random effects $\mathbf{b}_i$, thus can be written as $g = \mathbf{X}_i\boldsymbol{\beta} + \mathbf{Z}_i\mathbf{b}_i$. Hence, the response vector $\mathbf{y}_i$ of the i-th experimental unit is given by

$$\mathbf{y}_i = \mathbf{X}_i\boldsymbol{\beta} + \mathbf{Z}_i\mathbf{b}_i + \boldsymbol{\varepsilon}, \quad i = 1, \cdots, n, \tag{2}$$

and this expression sets a linear mixed effects model. In this case, the covariance structure is given by

$$\mathbf{Z}_i\mathbf{G}\mathbf{Z}_i^T + \mathbf{R}_i \tag{3}$$

with $\mathbf{Z}_i$ being the specification matrix of random effects. Besides that, $\mathbf{G}$ and $\mathbf{R}_i$ are the covariance matrix of random effects $\mathbf{b}_i$ and the random errors $\boldsymbol{\varepsilon}_i$, respectively. The covariance structure describes the variability behavior among the observations repeated within each profile and, in particular, its main diagonal represents the variability among different profiles. Now, also consider that such model declares time as a predictive variable X and has random effects on the intercept and on the slope of the linear function g, i.e.,

$$y_{ij} = (\beta_0 + b_{0i}) + (\beta_1 + b_{1i})x_{ij} + \varepsilon_{ij}, \quad j = 1, \cdots, p_i, \tag{4}$$

where y_{ij} represents the response from the i-th experimental unit observed at the j-th time and ε_{ij} is the error associated with such response. Furthermore, β_0 and β_1 are the fixed effects and describe the mean response shared by all experimental units. Therefore, by adding the random effect b_{0i} at the intercept β_0, one wishes to incorporate to the model, the difference between the mean intercept and the specific intercept of the i-th experimental unit. In general, this variability among experimental units is due to unweighted factors at the analysis, like genetic factors for instance (DIGGLE et al., 1994; MOLENBERGHS and VERBEKE, 2001). Similarly, by adding the random effect b_{1i} at the slope β_1, one wishes to identify the particular slope offset of the i-th experimental unit with respect to the mean slope shared by all units.

It is important to note that the slope β_1 of the linear function g is interpreted as the rate of change of the response variable y_{ij} regarding the predictive variable x_{ij}. It expresses the change suffered by the y_{ij} variable as the time x_{ij} changes. With the addition of the random effect, $(\beta_1 + b_{1i})$ is interpreted as the rate of change over time specific of the i-th experimental unit. Remember that the response profile of each experimental unit is split in two moments, before and after the intervention and, in each moment will be fitted a linear mixed effects model, as shown in Figure 1. Then, each response profile will contain two rates of change: $(\beta_{1b} + b_{1b_i})$ which denotes the rate of change of the response over time before the intervention, and $(\beta_{1a} + b_{1a_i})$ which represents the rate of change of the response over time after the intervention, see Table 1.

Table 1 - Rates of change over time of n longitudinal profiles, before and after an intervention

Experimental Unit	Before	After
1	$\beta_{1b} + b_{1b_1} = \beta'_{1b_1}$	$\beta_{1a} + b_{1a_1} = \beta'_{1a_1}$
2	$\beta_{1b} + b_{1b_2} = \beta'_{1b_2}$	$\beta_{1a} + b_{1a_2} = \beta'_{1a_2}$
$\vdots$	$\vdots$	$\vdots$
n	$\beta_{1b} + b_{1b_n} = \beta'_{1b_n}$	$\beta_{1a} + b_{1a_n} = \beta'_{1a_n}$

Therefore, to verify the influence of an intervention in the longitudinal response profile of the i-th experimental unit is equal to verify if there is any statistical

difference between the rates of change of the linear mixed effects models fitted before and after the intervention. It is important to clarify that this rates are incorporated in the particular effect of the i-th experimental unit, represented by adding the random effect. In other words, one wishes to evaluate the discrepancy between the parameters $\beta_{1b}^{'}$ and $\beta_{1a}^{'}$, defined at Table 1.

Moreover, if Z is a normal random variable, such that $Z \sim N(\mu, \sigma^2)$, then $(Z + c) \sim N(\mu + c, \sigma^2)$, with c as any constant. With these considerations and knowing that the random effects $\mathbf{b}_i \sim N_q(\mathbf{0}, \mathbf{G})$, one may conclude that the estimators of $\beta_{1b}^{'}$ and $\beta_{1a}^{'}$ are normal variables, being β_{1b} and β_{1a} constants. Therefore, the verification of the existence of statistical difference among the slopes can be performed using the paired t student test, being H_0 the hypothesis to be tested and H_1 the alternative declared as,

$$H_0 : \beta_{1b}^{'} = \beta_{1a}^{'}$$
$$H_1 : \beta_{1b}^{'} \neq \beta_{1a}^{'}.$$

To not reject H_0 means that the rates of change before and after the intervention are not statistically different, given the observed variable behavior y_{ij} is unchanged by the intervention. This means that, over time, the behavior of the response variable followed the expected course in the absence of interventions. However, to reject H_0 means that the rates of change before and after the intervention are statistically different, i.e., the expected behavior of the variable y_{ij} over time x_{ij} is influenced by the presence of the intervention.

The proposed methodology is still valid when g is a nonlinear function of fixed effects β and random effects $\mathbf{b}_i$, originating a nonlinear mixed model. The nonlinear relationship between the observed variable Y and the predictive variable time X is based on the mechanism producing the responses, having, usually, a physics or biological interpretation (PINHEIRO and BATES, 2000). Nonlinear mixed effects models are described in two hierarchical stages (LINDSTROM and BATES, 1990), which relates the variability between and within experimental units, respectively. To summarize, at first stage, the response y_{ij} belonging to the i-th experimental unit are modeled as

$$y_{ij} = g(\boldsymbol{\phi}_i, x_{ij}) + \varepsilon_{ij}, \quad j = 1, \cdots, p_i, \tag{5}$$

with g being a nonlinear derivative function of the parameters vector $\boldsymbol{\phi}_i$ and the predictive variable time x_{ij}. The vector $\boldsymbol{\phi}_i$ defines the parameters of each experimental unit, including fixed and random effects, so that $\boldsymbol{\phi} = (\phi_{0i}, \phi_{1i}, \cdots, \phi_{mi})$, being $m + 1$ the amount of parameters in the model. At the second stage, the vector of specific parameters $\boldsymbol{\phi}_i$ is modeled as

$$\boldsymbol{\phi}_i = A_i\boldsymbol{\beta} + B_i\mathbf{b}_i \tag{6}$$

where the matrix A_i and B_i with dimensions $(p_i \times t)$ and $(p_i \times q)$ are the specification matrix of fixed and random effects, respectively. Therefore, the nonlinear function g can be written as $g(A_i\boldsymbol{\beta} + B_i\mathbf{b}_i, x_{ij})$. Returning to the methodology, to verify

the influence of an intervention at the response variable y_{ij} is equal to verify if there is statistical difference between the rates of change ϕ_{1b_i} and ϕ_{1a_i}, regarding the nonlinear mixed effects models fitted at the experimental unit profile i before and after the intervention, analogous to the linear case described previously. It is important to clarify that the behavior of the variable will remain the same before and after the intervention, the change will occur in the intensity on which this behavior happens over time.

1.2 CD4 cells count

A total of 12 patients were selected from a special service program destined to people with sexual transmitted diseases/AIDS from Maringá, Paraná, Brazil, between 2014 and 2015. This patients had their CD4 cells count registered while visiting this services centers. Between the visits, a 30 day period, the patients received daily a glutamine based food supplement, and in the initial and final moments of this period, the CD4 cells count was registered. Hence, each patient has a response profile composed with the CD4 cells count, being the last but one count the representative of the initial moment of the diet, and the last count being the end of this intervention. Therefore, we would like to answer the following question: Does the glutamine based supplement promoted the increase of CD4 cells count in HIV-positive patients?

Above all, it is important to emphasize that each response profile have multiple counts of CD4 cells before the intervention and only one after the glutamine based supplementation. Hence, the mixed models fitted after the intervention used the two last observations of each profile. Besides that, it is common in HIV-positive patients the increase of CD4 cells from the moment they enter the health public program and started the antiretroviral cocktail treatment. As the increase behavior in the CD4 cells count is expected, to verify the influence of the glutamine diet in the increase of these cells is equal to verify the existence of statistical difference between the growth trends before and after the intervention.

One may note that it would be possible to use a paired t test to compare two CD4 cells count of a patient, measurements before and after the glutamine supplementation. However, to verify the existence of significant difference using isolated measurements from the response profile could lead to wrongful results, since this difference could be associated with the patient admittance into the health public program and not the intervention itself. The proposed methodology comes to remedy this uncertainty, using all data from before and after the intervention in the individual response profile to fit two mixed effects model, assigning the existence of significant difference exclusively to the intervention.

Hence, two linear mixed models, with random intercept and slope, were fitted to the CD4 cells count before and after the glutamine based supplementation. In both models, it was considered the time as a predictive variable and, as baseline, the first visit to the admittance site, i.e., the mean value of CD4 cells at the time $x = 0$. Therefore, the first model is expressed by,

$$y_{b_{ij}} = (\beta_{0b} + b_{0b_i}) + (\beta_{1b} + b_{1b_i})x_{b_{ij}} + \varepsilon_{b_{ij}}, \tag{7}$$

where $y_{b_{ij}}$ represents the response from the i-th patient at the j-th time and $(\beta_{0b} + b_{0b_i})$ and $(\beta_{1b} + b_{1b_i})$ denote the intercept and slope for the i-th patient before the glutamine intervention, respectively. Hence, the second model is written as,

$$y_{a_{ij}} = (\beta_{0a} + b_{0a_i}) + (\beta_{1a} + b_{1a_i})x_{a_{ij}} + \varepsilon_{a_{ij}}, \tag{8}$$

where $y_{a_{ij}}$ represents the response from the i-th patient at the j-th time and $(\beta_{0a} + b_{0a_i})$ and $(\beta_{1a} + b_{1a_i})$ denote the intercept and slope for the i-th patient after the intervention, respectively. Therefore, the inclusion of random effects at the intercept has the intent of incorporate the initial CD4 cells count variability among patients. As for the inclusion of the random effect at the predictive variable time, it represents the variability among patients observed over time x_{ij}, given that each patient has a peculiar slope. However, once the response profile of the i-th patient is analyzed individually, the effect between patients is filtered, such as the observed variability at the individual profile comes exclusively from the glutamine supplement, central point of the analysis.

Initially, the square root transformation for the CD4 cells count variable, which is approximately normal, was considered (BROWN and PRESCOTT, 2014; RIZOPOULOS, 2012). Posteriorly, the Poisson distribution was used to describe the dataset. The verification of statistical difference is performed with the paired Student t test, being verified and assumed the normality e homoscedasticity assumptions. Thus, the hypothesis to be tested H_0 and the alternative hypothesis H_1 is given by,

$$\begin{aligned} H_0 &: \beta_{1b}^{'} = \beta_{1a}^{'} \\ H_1 &: \beta_{1b}^{'} < \beta_{1a}^{'}. \end{aligned}$$

Performing the test at a 0.05 significance level, the descriptive level obtained was lower than 0.01 both for the normal model as for the Poisson model. The estimative values, as well as the model significance, are shown in Table 2. Hence, there are evidences that the glutamine supplement influenced the growth trend of the observed variable, promoting an increase in the CD4 cells count higher then expected over time, assuming the absence os interventions. Therefore, from the analysis performed, there are evidences that de CD4 cells count, in average, has increased after the intervention with the glutamine based diet.

Table 2 - Mixed models parameters before and after for the CD4 cells count (Normal and Poisson) and body mass of premature infants

Model	Parameter	CD4 cells count		Body mass
		Normal	Poisson	Normal
Before	β_{0b}	14.7255^{**}	5.2086^{**}	1000.7290^{**}
	β_{1b}	0.0014^{NS}	0.0004^{**}	0.0156^{**}
After	β_{0a}	15.8681^{**}	5.4611^{**}	973.1823^{**}
	β_{1a}	0.0592^{*}	0.0074^{**}	0.0153^{**}

$^{**}\ p-value < 0.01$; $^{*}\ p-value < 0.05$; $^{NS}\ p-value > 0.05$.

1.3 Body mass of preterm newborns

The body mass evolution of the newborns on its firsts days can be used as an indicator of food suitability (GARTNER *et al.*, 2005). Considering this, body mass measurements of eight preterm newborns were registered over time, in order to prove the efficiency of a feeding intervention. Over the first days of life, all children were fed with breast milk, LM, and, after a few days, they were fed with commercial dietary supplement ($FM85$) added to the breastmilk. The point of change in the diet was peculiar to each child, however all received both diet in the same order. Besides that, as newborn lose, in average, $\sim 5-7\%$ of body mass during its firsts $2-3$ days of life (BERTINI *et al.*, 2015), the lowest mass over the evaluation period was taken as the initial measurement in each child profile. Therefore, the response profile of the i-th newborn is composed by several measurements of body mass before and after the diet intervention, being the initial value correspondent to the lowest measure. In this circumstances, we wish to answer the following question: does breastmilk added from commercial dietary supplement promote an evolution in the preterm newborn body mass equivalent only breast milk diet?

To answer this question, the proposed methodology was applied, whose notoriety is due to the usage of all repeated observations to identify significant differences in the response profile of each child towards the intervention. The body mass evolution of newborns from the fourth day of life is predicted and, consequently, the growth trend of the rate of change in the body mass is expected. Hence, to verify the influence of the food supplement in the increase of body mass in preterm newborns is equal to verify if there is statistical difference between the growth trend promoted by the breast milk and by the commercial food supplement over time.

It is essential to consider that the rate of change of the body mass y_i of each child over time x, dy_i/dx, is proportional to the child's body mass in a given instant x_{ij}. Which means that, bigger the child's body mass, bigger will be its variation. Thus, by solving the differential equation $dy_i/dx = ky_i$, being k a proportionality constant e considering that the initial time is that of which the child has reached the lower mass value, β_{0i}, we obtain that $y_i(x) = \beta_{0i}\exp(\beta x)$, being β_{0i} a parameter

to be estimated. Therefore, the nonlinear relationship based on the mechanism producing the responses is established between the rate of change of newborns body mass and time.

Hence, two nonlinear mixed effects models were fitted with the intent to model the growth trends of newborns body mass resulting in the diets based on breast milk and the commercial food supplement. In both, time is declared as a predictive variable and the lower registered mass as baseline. Thus, the responses $y_{b_{ij}}$ and $y_{a_{ij}}$ of the i-th newborn observed in the j-th time before and after the commercial food supplement, respectively, are modeled as

$$y_{b_{ij}} = \phi_{0b_i} \exp(\phi_{1b_i} x_{ij}) + \varepsilon_{b_{ij}} \tag{9}$$

and

$$y_{a_{ij}} = \phi_{0a_i} \exp(\phi_{1a_i} x_{ij}) + \varepsilon_{a_{ij}}, \tag{10}$$

where the vectors $\boldsymbol{\phi_{b_i}}$ e $\boldsymbol{\phi_{a_i}}$ of the specific effects of the newborn i before and after the intervention are expressed by,

$$\boldsymbol{\phi}_{b_i} = \begin{bmatrix} \phi_{0b_i} \\ \phi_{1b_i} \end{bmatrix} = \begin{bmatrix} \beta_{0b} \\ \beta_{1b} \end{bmatrix} + \begin{bmatrix} b_{0b_i} \\ b_{1b_i} \end{bmatrix}$$

and

$$\boldsymbol{\phi}_{a_i} = \begin{bmatrix} \phi_{0a_i} \\ \phi_{1a_i} \end{bmatrix} = \begin{bmatrix} \beta_{0a} \\ \beta_{1a} \end{bmatrix} + \begin{bmatrix} b_{0a_i} \\ b_{1a_i} \end{bmatrix}$$

where ϕ_{0b_i} and ϕ_{1b_i} expresses the initial body mass and rate of change specific to the i-th newborn before the intervention. Analogously, ϕ_{0a_i} and ϕ_{1a_i} expresses the body mass and the rate of change specific to the i-th newborn after the intervention.

The verification of the statistical difference between the growth trends of the rate of change of the body mass is performed by the paired Student t test, assuming the normality and homoscedasticity were verified. Thus, the hypothesis to be tested H_0 and the alternative hypothesis H_1 are,

$$H_0 : \beta_{1b}^{'} = \beta_{1a}^{'}$$
$$H_1 : \beta_{1b}^{'} \neq \beta_{1a}^{'}.$$

Performing the test with a 0.05 significance level, the descriptive level value was ~ 0.7. Therefore, there are evidences that the commercial food supplement did not influenced the growth trend of the rate of change of the body mass in preterm newborns. Regardless of, such evidences suggests that the commercial food supplement sustained the body mass growth trend equally to the breast milk, as the tested hypothesis was not rejected. Hence, according to the data observed in this study, the commercial food supplement can be suggested as an alternative diet for preterm newborns give the lack of breast milk.

We can observe this results at Figure 2. The plot below in the right presents the behavior of marginal profiles for the moments the children were fed with LM and $FM85$. Both curves were fitted since the first experimental day until 65 days,

which is equivalent to the time of the child with most number of observations. The other three graphs presents three conditional profiles and the data observed for three children in the experiment. The vertical dotted line is equivalent to the moment the children changed its food supply.

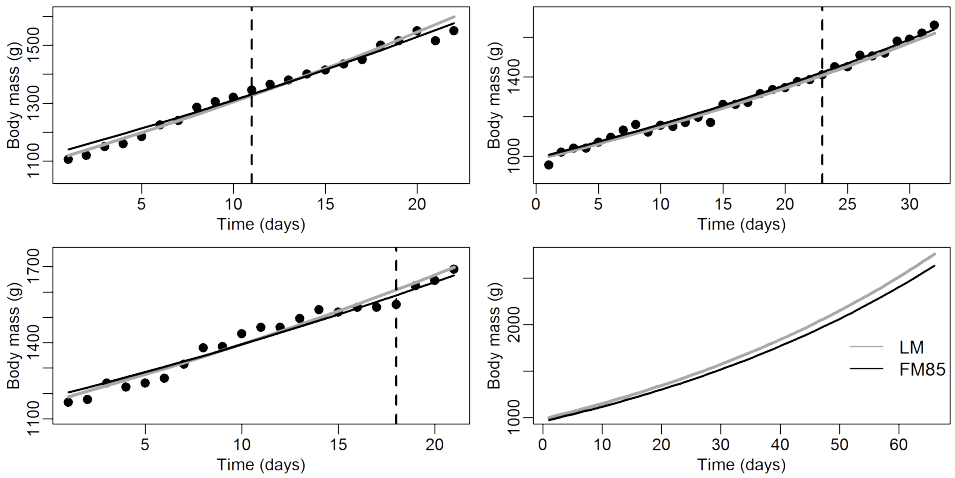

Figure 2 - Longitudinal profile for three children fed with LM and $FM85$ and the mean profile for both types of food supply. The below and in the right graph presents the mean fit for LM and $FM85$. The other refers to the observed data of three children and its conditional profiles fitted for LM and $FM85$. The vertical dotted line presents the moment each child changed its food supply, from LM to $FM85$.

2 Final considerations

The proposed methodology solves the questions regarding interventions performed in longitudinal profiles considering the existent correlation in the data. By using two regression in the same individual profile, we showed to be possible and, statistically advisable, the identification of a significant difference between its rates of change.

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- *RESUMO: Por vezes, o comportamento de uma variável resposta acompanhada ao longo do tempo pode ser influenciado por uma intervenção realizada durante o período experimental. Nessa perspectiva, o presente estudo propõe uma metodologia simples baseada no ajuste de dois modelos de efeitos mistos em perfis longitudinais, antes e depois de uma intervenção, para a verificação de diferenças significativas. A notoriedade dessa proposta consiste do emprego de todas as observações repetidas da variável resposta na comparação perante a intervenção. A proposta dessa metodologia foi motivada por dois conjuntos de dados reais. Modelos lineares de efeitos mistos foram ajustados no primeiro conjunto de dados, o qual refere-se a contagem de células CD4 em pacientes com HIV positivo que, durante 30 dias consecutivos, receberam uma suplementação alimentar com glutamina. No segundo conjunto, modelos não lineares de efeitos mistos foram ajustados as medições da massa corporal de recém nascidos prematuros, cuja dieta alimentar inicial baseou-se no leite materno, sendo posteriormente substituído por um suplemento alimentar comercial. A metodologia proposta foi capaz de detectar diferença na tendência de crescimento da contagem de células CD4 após a suplementação com glutamina nos perfis dos pacientes observados. Além disso, forneceu evidências que sugerem o suplemento alimentar comercial como alternativa ao leite materno na dieta de recém nascidos prematuros, por manter a tendência de crescimento da massa corporal.*

- *PALAVRAS-CHAVE: Modelos mistos lineares e não lineares; perfil longitudinal; recém nascido prematuro; leite materno; HIV; glutamina.*

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