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Piecewise nonlinear mixed-effects models for modeling cardiac function and assessing treatment effects

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Abstract

Mixed-effects model is an efficient tool for analyzing longitudinal data. The random effects in a mixed-effects model can be used to capture the correlations among repeated measurements within a subject. Mixed effects model can be used to describe individual response profile as well as population response profile. In this manuscript, we apply mixed-effects models to the repeated measurements of cardiac function variables including heart rate, coronary flow, and left ventricle developed pressure (LVDP) in the isolated, Langendorff-perfused hearts of glutathione s-transferase P1/P2 (*GSTP*) gene knockout and wild-type mice. Cardiac function was measured before and during ischemia/reperfusion injury in these hearts. To describe the dynamics of each cardiac function variable during the entire experiment, we developed piecewise nonlinear mixed-effects models and a change point nonlinear mixed effect model. These models can be used to examine how cardiac function variables were altered by ischemia/reperfusion-induced injury and to compare the cardiac function variable between genetically engineered (null or transgenic) mice and wild-type mice. Hypothesis tests were constructed to evaluate the impact of deletion of *GSTP* gene for different cardiac function variables. These findings provide a new application for mixed-effects models in physiological and pharmacological studies of the isolated Langendorff-perfused heart.

Keywords

Mixed-effects model; Piecewise nonlinear mixed-effects model; Change point model; Cardiac function; Ischemia-reperfusion

1. Motivation

Myocardial ischemia/reperfusion (I/R) injury has resulted in significant morbidity and numerous studies have been carried out to discover endogenous mechanisms to protect the

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heart against I/R injury. Glutathione s-transferase P (GSTP) has been identified as an antioxidant cardiac enzyme that protects against I/R injury by catalyzing the removal of toxic lipid peroxidation products [1]. In an isolated heart, cardiac function is monitored over time and function is characterized by heart rate (i.e., the number of beats per minute (bpm)), coronary blood flow (mL/min), and left ventricle developed pressure (LVDP) (i.e., the difference of left ventricle systolic pressure and left ventricle diastolic pressure; mmHg). To examine whether GSTP gene protects against I/R injury, the isolated, Langendorff-perfused hearts from GSTP-null mice (KO) and wild type mice (WT) were exposed to 30 min of ischemia followed by 45 min of reperfusion [1]. The cardiac function variables (heart rate, coronary blood flow, and LVDP) were measured continuously including 5 min pre-ischemia, 30 min of ischemia, and 45 min of reperfusion. The data were summarized in 1 min intervals. Heart rate, coronary blood flow, and LVDP are shown, respectively, in Fig. 1 Panels A1–A3 for wild-type mice ($n = 7$) and in Fig. 1 Panels B1–B3 for GSTP-null mice ($n = 6$). Typically, the Student's t -test is used to test whether two groups are the same at each time point [2] or a repeated measures analysis of variance (rANOVA) is applied to test whether there are group difference, time effect, and group by time interaction [3]. The number of repeated observations for each subject is 80, and thus, the t -test without multiplicity adjustment seems inappropriate. The rANOVA requires that all individuals have a complete balanced data and a fixed time schedule, and the rANOVA treat time points as different levels of a factor variable. Because each cardiac function variable was recorded in 80 min interval, and each cardiac function variable changes dramatically over the three treatment periods (i.e., pre-ischemia, ischemia, and reperfusion), the application of rANOVA was limited. Thus, a more flexible model, mixed-effects model, seems to be more appropriate. In the mixed-effects model time points are not necessary fixed, time is treated as a continuous variable, and all available data can be used in mixed-effects model provided that data are missing at random [4]. In a mixed-effects model, the correlation between repeated measurements is captured by random effects [3]. Moreover, mixed-effects models provide a more flexible covariance structure for non-constant within-subject correlation [5]. Several studies have suggested that a mixed-effects model is very useful in biomedical research and especially in analyzing complex data with multi-source variance [6]. In many longitudinal analyses, change is assumed to be linear but in numerous situations, the change is not uniform but rather faster during some periods and slower in others [7]. In these cases, nonlinear mixed-effects (NLME) models or piecewise polynomials are more appropriate than linear mixed-effects (LME) models to describe these nonlinear changes. However, nonlinear models incorporate characteristics of the data, such as asymptotes and monotonicity; the parameters in nonlinear models are often interpretable; and nonlinear models often provide more reliable predictions for the response variable outside the observed range of the data than, say, polynomial models would [8]. Thus, in this paper, we applied NLME models to investigate the dynamics of the cardiac function variables.

For hearts undergoing ischemia/reperfusion injury, cardiac function during pre-ischemia, ischemia, and reperfusion periods is quite different, and the change in function during each period is often nonlinear. To describe the heart rate and coronary blood flow for the entire experiment, we developed a nonlinear mixed model for each period and a piecewise nonlinear mixed model over the entire experiment. LVDP, the difference between left

ventricle systolic pressure and left ventricle diastolic pressure, is an important variable to characterize cardiac function, and LVDP decreased to very low values during ischemia, and gradually increased during reperfusion. The elapsed time before there is an increase in the LVDP is inversely related to cardiac recovery, and a shorter elapsed time indicates a less injured heart. The comparison of LVDP between pre-ischemia and the recovery during reperfusion is not our interest. Therefore we only focus on modeling LVDP during reperfusion. The elapsed time from a constant phase to an increasing phase is often called a change point. Note that the elapsed times will differ for different subjects, and thus, we proposed a random change point nonlinear mixed-effects model for studying LVDP during reperfusion. In both the nonlinear mixed effect models or change point nonlinear mixed effects models, the parameters for each subject are expressed as the summation of group level parameters (i.e., fixed effects) and the deviation of the subject parameters away from its group level parameters (i.e., random effects). In Section 2.1, we provide a brief introduction to nonlinear mixed effect models, including parameter estimation and statistical inferences. In Section 2.2, we provide a general framework for change point mixed effect model. In Section 3, we develop nonlinear mixed effect models for examining heart rate and coronary blood flow, and the change point nonlinear mixed effects models for examining LVDP. In Section 4, we present the analytic results for the experiment data shown in Fig. 1. In Section 5, we carried out simulations to examine the performance of the estimation of parameters when the number of subjects is small and moderate. The last section provides relevant discussion of models and their potential applications.

2. General nonlinear mixed effect models and change point model

2.1. General nonlinear mixed-effects (NLME) model

NLME models are often used because these models provide a parsimonious description of the data and reliable predictions for the response variables [8]. The general formula of a single level NLME model is proposed by Lindstrom and Bates [5]. At the first level the j th observation on the i th subject is modeled as:

$$y_{ij} = f(\phi_i, t_{ij}) + \varepsilon_{ij} \quad (1)$$

where $i = 1, \dots, N$, $j = 1, \dots, n_i$, and $\varepsilon_{ij} \sim N(0, \sigma)$. N is the number of subjects, n_i is the number of observations on the i th subject, f is a real-valued function of a subject-specific parameter vector ϕ_i and a covariate vector t_{ij} , and ε_{ij} is a normally distributed within-subject error term. The function f is nonlinear in at least one component of the subject-specific parameter vector ϕ_i , which is modeled as:

$$\phi_i = A_i \beta + B_i b_i, \quad b_i \sim N(0, D) \quad (2)$$

where β is a p -dimensional vector of fixed effects parameters, b_i is a q -dimensional random effects vector associated with i th subject, and the matrices A_i and B_i are of appropriate dimensions and depend on the i th subject and possibly on the values of some covariates. Let

us denote $\varepsilon_i = (\varepsilon_{i1}, \dots, \varepsilon_{in_i})^T$, and $b_i = (b_{i1}, \dots, b_{iq})^T$. It is generally assumed that (i) ε_i has normal distribution with mean zero and variance $\sigma^2 I$, (ii) b_i has a normal distribution with mean zero and variance–covariance matrix D , and (iii) ε_i and b_i are independent ($i = 1, \dots, N$) [8]. Here we assume that subjects' responses follow a similar function form with parameters varying among subjects. The parameters for i th subject are the vector ϕ_i , which composite the fixed effect parameter β and random effects b_i . The response function determined by fixed effect may be considered the average response for the population, and the response function determined by ϕ_i may be considered the response function for i th subject. The diagonal entries of the variance–covariance matrix D , say $\sigma_1^2, \dots, \sigma_q^2$, reflect the variation of the random effects, indicating the between-subject variation. The off-diagonal entries, such as $\rho_{kk'} \rho_k \rho_{k'}$, describes the covariance between b_{ik} and $b_{ik'}$, where $\rho_{kk'}$ is the correlation coefficient between b_{ik} and $b_{ik'}$. The matrix D could be specified as different forms, such as positive definite matrix, compound symmetric matrix, or a blocked diagonal matrix [3,8]. Let us denote θ as the parameters specifying D , that is, θ includes all parameters which capture $\sigma_1^2, \dots, \sigma_q^2$ and $\rho_{kk'}$ ($1 \leq k < k' \leq q$). The θ specifying D and σ^2 specifying the within-subject error are often called variance-covariance component parameters. The estimation of the fixed effect parameter β and the variance–covariance component parameters θ and σ^2 can be obtained by maximizing the marginal distribution for y , which generally does not have a closed-form expression. To obtain the solutions for maximizing the marginal distributions, different approximations have been proposed. The *nlme* package in R (<http://www.r-project.org/>) is implemented by taking a first-order Taylor expansion of the model function f around the expected value of the random effects [5,8]. The *nlme* function in this package provides the estimates for β and the variance–covariance component parameters θ and σ^2 , the asymptotic variance for $\hat{\beta}$, and the 95% confidence intervals for all parameters. In addition, the predicted values for \hat{b}_i ($i = 1, \dots, N$) can also be obtained. Subsequently, one can obtain the response profile at the population level as $f(A_i \hat{\beta}, t)$ and at the subject-level as $f(A_i \hat{\beta} + B_i \hat{b}_i, t)$ for i th subject, and their variances by using the δ -method [9,10]. The statistical inferences for the estimable function of β could be carried out by using the Wald's statistics or the likelihood ratio tests (LRT) [8,9]. We started with a full model that included all parameters and D as a blocked diagonal matrix. To obtain a parsimonious model, we first removed the least significant variance-covariance components, and then removed the least significant fixed effects parameter. The LRT and the Akaike Information Criterion (AIC) [11] were applied to obtain a parsimonious model for interpretation and statistical inferences.

2.2. Change point nonlinear mixed effect model

The change point nonlinear mixed effect model still has the general form (1), that is, $y_{ij} = f(\phi_i, x_{ij}) + \varepsilon_{ij}$ with $\varepsilon_{ij} \sim N(0, \sigma^2)$. However, the response variable for i th individual often includes K different phases. The first phase can be described as $f_1(\phi_{i1}, t)$ for $t < \gamma_{i1}$, the k th ($k = 2, \dots, K-1$) phase could be described as $f_k(\phi_{ik}, t)$ for $\gamma_{i(k-1)} < t < \gamma_{ik}$, and the K th phase could be described as $f_K(\phi_{iK}, t)$ for $t > \gamma_{i(K-1)}$. Here γ_{ik} ($k = 1, \dots, K-1$) are often called the change point for i th subject from k th phase to $(k+1)$ th phase. When $K = 2$, the expected response profile can be expressed as:

$$f(\phi_i, t) = \begin{cases} f_1(\phi_{i1}, t) & \text{if } t \leq \gamma_i \\ f_2(\phi_{i2}, t) & \text{if } t > \gamma_i \end{cases} \quad (3)$$

Generally, we assume that the response profile is continuous at the change point γ_i , that is, $f_1(\phi_{i1}, \gamma_i) = f_2(\phi_{i2}, \gamma_i)$. The individual response parameters (i.e., $\phi_i = (\phi_{i1}, \phi_{i2}, \gamma_i)$) are expressed by the summation of fixed effects and random effects: $\phi_i = A_i\beta + B_i b_i$, as shown in Eq. (2). Cudeck and Klebe [12] illustrated the application of change point models to psychology, and implemented the algorithm in SAS. Pearson et al. [13] used a smoothing function in the change point model to smooth the response function at the change point. In this manuscript, we assume that the response function is continuous at the change point, and used the *nlme* package in R to obtain the estimates for the involved parameters. Model selection, parameter estimation, and statistical inferences follow the same principle as in the nonlinear mixed effect models mentioned in the previous section.

3. Piecewise nonlinear mixed effect models and change point model for cardiac function variables

In this section, we applied exponential growth function of the form $y = A(1 - e^{-kt})$ (where $t > 0$ and $k > 0$) [8] to describe the dynamics of heart rate and LVDP during reperfusion, and applied exponential decaying function of the form $y = Ae^{-kt}$ (where $t > 0$ and $k > 0$) to describe the heart rate during ischemia. We applied the first-order compartment model along with the exponential growth function to describe the coronary flow during reperfusion [8,13]. The parameters in these models incorporate theoretical characteristics of the data and are often easy to interpret.

3.1. Piecewise nonlinear mixed-effects model for heart rate

As illustrated in Fig. 1 (Panels A1, B1), heart rate does not change significantly during pre-ischemia in either WT mice or KO mice, declines exponentially during ischemia, and then increases gradually reaching a stable plateau during reperfusion. We propose using a constant (say ϕ_{i1}) to describe the heart rate in pre-ischemia, an exponential decaying function of the form $\phi_{i1} \exp(-\exp(\phi_{i2})(t - 5)_+)$ to describe the heart rate during ischemia, and exponential growth function of the form $\phi_{i3}[1 - \exp(-\exp(\phi_{i4})(t - 35)_+)]$ to describe the heart rate during reperfusion. The step function x_+ is defined as x if $x > 0$, and zero otherwise. The step functions $(t - 5)_+$ and $(t - 35)_+$, respectively, are the time since the start of ischemia and the time since the start of reperfusion. The heart rate for i th subject during the entire experiment can be described as:

$$f(\phi_i, t) = \begin{cases} \phi_{i1} & 0 < t \leq 5 \\ \phi_{i1} \exp(-\exp(\phi_{i2})(t - 5)_+) & 5 < t \leq 35 \\ \phi_{i3}[1 - \exp(-\exp(\phi_{i4})(t - 35)_+)] & 35 < t \leq 80 \end{cases} \quad (4)$$

The parameter for i th subject, say $\phi_i = (\phi_{i1}, \phi_{i2}, \phi_{i3}, \phi_{i4})$, depends on its group assignment (i.e., WT or KO mice) as well as the deviation from group-level parameters. That is,

$$\phi_{ik} = \beta_{k1} + \beta_{k2} type_i + b_{ik} \quad (k=1, \dots, 4) \quad (5)$$

and

$$type_i = \begin{cases} 1 & \text{if subject } i \text{ is KO mouse} \\ 0 & \text{if subject } i \text{ is WT mouse} \end{cases} \quad (6)$$

Model (4) implies that the function is continuous during the entire experimental period. For example, the heart rate during pre-ischemia is ϕ_{i1} , and the heart rate at the beginning of ischemia is also ϕ_{i1} since $\phi_{i1} \exp(-\exp(\phi_{i2})(t-5)_+)$ goes to ϕ_{i1} when t goes to 5 from right. The heart rate declines to zero during ischemia, and recovers during reperfusion and tends to stabilize at a value ϕ_{i3} during the later phase of reperfusion. The $\exp(\phi_{i2})$ is the decline rate constant during ischemia, and $\exp(\phi_{i4})$ is the increasing rate constant during reperfusion. The group-level response profile for WT mice is characterized by the parameters β_{k1} ($k=1, \dots, 4$), and obtained by replacing ϕ_{ik} with β_{k1} ($k=1, \dots, 4$) in Eq. (4). The group-level response profile for KO mice is characterized by the parameters $\beta_{k1} + \beta_{k2}$ ($k=1, \dots, 4$), and obtained by replacing ϕ_{ik} with $\beta_{k1} + \beta_{k2}$ ($k=1, \dots, 4$) in Eq. (4). The response profile for i th subject is characterized by the summation of its group-level parameter, i.e., $\beta_{k1} + \beta_{k2} type_i$ (fixed effects), and the deviation from its group-level parameter, i.e., b_{ik} ($k=1, \dots, 4$) (random effects). The difference between WT and KO can be characterized by β_{k2} ($k=1, \dots, 4$). In addition, β_{11} and $\beta_{11} + \beta_{12}$, respectively, describe the average heart rate for KO and WT hearts in pre-ischemia, and β_{31} and $\beta_{31} + \beta_{32}$, respectively, describe the stabilized heart rate during reperfusion for WT and KO mice. It is straight forward to test whether the heart rate during reperfusion is recovered to pre-ischemia level for WT and KO mice, respectively, by testing $\beta_{31} - \beta_{11} = 0$ or $(\beta_{31} + \beta_{32}) - (\beta_{11} + \beta_{12}) = 0$.

3.2. Piecewise nonlinear mixed-effects model for coronary flow

Fig. 1 (Panels B1, B2) illustrates that coronary flow is close to a constant for WT mice and KO mice during pre-ischemia (say, ϕ_{i1}), drops to zero during ischemia, increases to a high peak shortly after the start of reperfusion, and then decreases gradually and plateaus at ϕ_{i2} during reperfusion. To describe the coronary flow for WT mice and KO mice during the entire experiment, we propose the following piecewise nonlinear mixed-effects model:

$$f(\phi_i, t) = \begin{cases} \phi_{i1} & 0 < t \leq 5 \\ 0 & 5 < t \leq 35 \\ \phi_{i2} - \phi_{i2} \exp(-\exp(\phi_{i4})(t-35)_+) + g(\phi_i, t) & 35 < t \leq 80 \end{cases} \quad (7)$$

where $g(\phi_i, t) = (\phi_{i5} \exp(\phi_{i3}) / \exp(\phi_{i4}) - \exp(\phi_{i3})) [\exp(-\exp(\phi_{i3})(t-35)_+) - \exp(-\exp(\phi_{i4})(t-35)_+)]$, and $\phi_i = (\phi_{i1}, \phi_{i2}, \phi_{i3}, \phi_{i4}, \phi_{i5})$, and $\phi_{ik} = \beta_{k1} + \beta_{k2} type_i + b_{ik}$ ($k=1, \dots, 5$) as shown in Eq. (5). The expression describing the coronary flow during reperfusion consists

two components: the first component is an exponential growth curve with asymptote at ϕ_{i2} , while the second component, say $g(\phi_i, t)$, is a first-order compartment model, capturing the bell-shaped curves for the coronary flow [8,14]. In Eq. (7), the fixed effects β_{11} and $\beta_{11} + \beta_{12}$ are, respectively, the means of the coronary flows for WT mice and KO mice in pre-ischemia; β_{21} and $\beta_{21} + \beta_{22}$ represent, respectively, the stabilized coronary flow for WT mice and KO mice during reperfusion. The random effects b_{ik} ($i = 1, \dots, N$) describe the deviation of coronary flow for i th subject away from its group-level parameters. Using the parameters specified in the model, we can directly examine whether coronary flow for WT mice and KO mice during each time period is different by examining whether β_{k2} is different from zero. In addition, using the current specification, we can examine whether coronary flow during reperfusion recovers to its pre-ischemia level by testing if $\beta_{21} - \beta_{11} = 0$ for WT mice and testing if $(\beta_{21} + \beta_{22}) - (\beta_{11} + \beta_{12}) = 0$ for KO mice.

3.3. Change point nonlinear mixed effect models for left ventricle developed pressure (LVDP)

Fig. 1 (Panels A3, B3) illustrates that the LVDP is constant in pre-ischemia, decreases to a low value during ischemia, stays at the low value until an onset of increasing phase of LVDP during reperfusion, increases gradually after onset to plateau in the later stage of reperfusion. The onset values and the stabilized values were different for different subjects. We introduced random effects for the onset values and the stabilized values. The onset value and the stabilized LVDP were critically important because these characterized how quickly and to what degree the LVDP recovered. In consideration of all these aspects, we proposed the following change point model to describe the LVDP during reperfusion:

$$f(\phi_i, t) = \begin{cases} \phi_{i1} & t \leq \phi_{i2} \\ \phi_{i1} + \phi_{i3} [1 - \exp(-\exp(\phi_{i4})(t - \phi_{i2})_+)] & t > \phi_{i2} \end{cases} \quad (8)$$

where $\phi_i = (\phi_{i1}, \phi_{i2}, \phi_{i3}, \phi_{i4})$, and $\phi_{ik} = \beta_{k1} + \beta_{k2} \text{ type}_i + b_{ik}$ ($k = 1, \dots, 4$). In model (8), ϕ_{i1} is a constant LVDP before the onset of the increasing phase for LVDP, ϕ_{i2} is the onset point (i.e., change point) from constant phase to the exponential growth phase, representing the unknown transition time between the constant LVDP and the exponential growth, $\phi_{i1} + \phi_{i3}$ is the stabilized status of the LVDP during reperfusion, and $\exp(\phi_{i4})$ is the constant growth rate during the exponential LVDP growth phase. The subject-level parameters ϕ_{ik} ($k = 1, \dots, 4$) are expressed as the summation of population parameters (i.e., $\beta_{k1} + \beta_{k2} \text{ type}_i$) and the deviation of the subject-level parameters away from their population parameters (i.e., b_{ik}). The population parameters are used to compare group differences, and the subject-level parameters are used to examine subject-level responses and between-subjects variations.

4. R-code and analysis results for cardiac function variables

We applied the proposed models to analyze the experimental data shown in Fig. 1. For each cardiac function variable, we started by fitting the full model, as proposed in Section 3, and we fitted several reduced models as well. The likelihood ratio tests (LRT) are applied to compare two nested models, and the LRT along with the AIC criterion are used to select a

parsimonious model as our final model. We first consider a simplified variance–covariance matrix by setting the insignificant components from the full model to zero. If the LRT for comparing the reduced model and full model was not significant, we considered a further reduced model which excluded least significant fixed effects parameters. Otherwise, we remained the variance–covariance parameters in the model and considered a reduced model on the fixed effects parameters. The estimated parameters and their 95% confidence intervals in the full model, the reduced variance–covariance components model, and the final model are reported in Tables 1–3 for different cardiac function variables, and the interpretation and inferences for each cardiac function variable are based on the final parsimonious models. The fitted subject-level and group-level response curves are, respectively, shown in Panels A and B in Figs. 2–4, where the pointwise 95% confidence intervals for subject-level response curve and group-level response curves, along with the observations, are also shown. The Wald test was applied to test whether the cardiac function during reperfusion recovered to its pre-ischemia level. The *nlme* and *anova* in the R-package “nlme” are the major functions to be used for the analyses. The data frame for each cardiac function variable includes type (i.e., WT or KO), subject (i.e., subject identification number), time (in minutes), cardiac function variable, and period (i.e., pre-ischemia, ischemia, and reperfusion). The main results and the key R-code for each cardiac function variable is presented under the following sections.

4.1. Analysis results for heart rate

We started from the full model (4) with a block diagonal variance–covariance matrix for random effects b_j , and the estimated parameters and their 95% confidence intervals are reported in Table 1 under the column title “Full model”. Since the correlation coefficients between b_{j1} and b_{j2} (i.e., ρ_{12}) was not significantly different from zero, we fitted a reduced model with ρ_{12} being zero, and the fitted results are reported in Table 1 under the column title “Reduced variance component model”. The LRT for this reduced model versus the full model was not significant (p -value = 0.932). In the reduced model, the fixed effects parameters β_{12} and β_{22} were not significantly different from zero, we further removed these two parameters, and the LRT indicated that the final resulting model was not significantly different from the full model (p -value = 0.639) as well as the reduced variance component model (p -value = 0.431). The estimated parameters and their 95% confidence intervals for this model are reported in Table 1 under the column title “Final Model”. The observations, the fitted subject-level and group-level response curves based on the final model, along with their point-wise 95% confidence intervals, were shown in Fig. 2 Panel A and B, respectively. Based on the estimated parameters (Table 1 under the column title “Final model”) and the fitted values (Fig. 2), we conclude that: (i) in pre-ischemia, the heart rates of WT mice and KO mice were not significantly different because β_{12} was not significantly different from zero, and the between subject variation counts 66% of total variation since $\sigma_1^2 / (\sigma_1^2 + \sigma^2) = 66\%$; (ii) during reperfusion, the average stabilized heart rate for KO mice was significantly higher than that of WT mice because β_{32} was significantly larger than zero, and the between-subject variation was large and accounts for approximately 92% of the total variation; (iii) during reperfusion, the increasing rate constant for KO mice was significantly smaller than for WT mice because β_{42} was significantly smaller than zero; (v) during reperfusion, the subject-level stabilized heart rate was negatively associated with the

increasing rate constant because the correlation between $b_{\beta 3}$ and $b_{\beta 4}$ (i.e., ρ_{34}) was significantly smaller than zero. The R-code for analyzing heart rate is shown as follows:

```
HeartRate <- function(time, phi1, phi2, phi3, phi4) { phi1 * (time <= 5) + phi1 * exp(-
  exp(phi2) * (time - 5)) * (time > 5 & time <= 35) + as.numeric(SSasymOrig((time - 35),
  Asym = phi3, lrc = phi4)) * (time > 35) }

HR.full.nlme <- nlme(Rate ~ HeartRate(time, phi1, phi2, phi3, phi4), na.action =
  na.omit,

  fixed = phi1 + phi2 + phi3 + phi4 ~ type,

  random = pdBlocked(list(pdSymm(phi1 + phi2 ~ 1), pdSymm(phi3 + phi4 ~ 1))),

  start = c(phi1 = 257.12, 20, phi2 = n1start[2], 0.1, phi3 = n2start[1], 200, phi4 = n2start[2], 1),
  data = pre.isch.per, method = "ML")
```

One can fit the reduced variance component model by replacing the “random” statement with “random = pdBlocked(list(pdDiag(phi1 + phi2 ~ 1), pdSymm(phi3 + phi4 ~ 1)))”, and fit the final model by additionally replacing the “fixed” statement with “fixed = list(phi1 + phi2 ~ 1, phi3 + phi4 ~ type)”.

We constructed test statistics to show if heart rate during reperfusion recovered to the pre-ischemic level for WT mice and KO mice. The associated null hypothesis for WT mice was $H_0: \beta_{11} - \beta_{31} = 0$, and the associated null hypothesis for KO mice was $H_0: \beta_{11} - (\beta_{31} + \beta_{32}) = 0$. Based on the final model, the Wald test statistic for WT mice was

$$T = (\hat{\beta}_{11} - \hat{\beta}_{31}) / \sqrt{\text{var}(\hat{\beta}_{11} - \hat{\beta}_{31})} = 0.571 \text{ with a } p\text{-value at } 0.568, \text{ indicating that the heart rate in pre-ischemia was not significantly different from the stabilized heart rate during reperfusion. The Wald test statistic for KO mice was}$$

$$T = (\hat{\beta}_{11} - \hat{\beta}_{31} - \hat{\beta}_{32}) / \sqrt{\text{var}(\hat{\beta}_{11} - \hat{\beta}_{31} - \hat{\beta}_{32})} = -2.731 \text{ with a } p\text{-value at } 0.006, \text{ indicating that the heart rate in pre-ischemic is significantly lower than the stabilized heart rate during reperfusion.}$$

4.2. Analysis of coronary flow

To examine coronary flow, we started from the full model (7) with a blocked diagonal matrix for random effects b_i . The estimated parameters and their 95% confidence intervals for the full model are reported in Table 2 under the column title “Full model”. From the results of the full model, all the correlation coefficients except the one between $b_{\beta 2}$ and $b_{\beta 5}$ (i.e., ρ_{25}) were not significantly different from zero. We fitted a reduced model with these variance components being zero, and the results are presented in Table 2 under the column “Reduced variance component model”. The AIC for the reduced model was smaller than the full model, and the LRT indicated that the two models were not significantly different (p -value = 0.07). Thus, the reduced model was preferred. Furthermore, we removed the least significant terms of fixed effects parameters. The LRT indicated that this model was not significantly different from the full model as well as the reduced variance component model. The results of the final model are reported in Table 2 under the column title “Final model”. The fitted subject-level coronary flow and group-level coronary flow are, respectively, shown in Panel

A and B (Fig. 3), along with their pointwise 95% confidence intervals and the observed coronary flow. Based on the estimated parameters (Table 2) and the fitted values (Fig. 3), we conclude that (i) in pre-ischemia, the coronary flow between WT mice and KO mice was not significantly different because β_{12} was not significantly different from zero, and the between-subject variation accounted for 97.8% of the total variation; (ii) during reperfusion, the average stabilized coronary flow for WT mice was significantly higher than in KO hearts because β_{22} was significantly less than zero, and the between-subject variation was large and accounted for approximately 97.5% of the total variation. The R-code for analyzing coronary flow is shown as follows:

```
flow.pd<-function(phi1,phi2,phi3,phi4,phi5,time){phi1*(time<=5)+(phi2-
phi2*exp(-exp(phi4)*(time-35))+phi5*exp(phi3)/(exp(phi4)-exp(phi3))*(exp(-
exp(phi3)*(time-35))-exp(-exp(phi4)*(time-35))))*(time>=35)}

flow.full.nlme<-nlme(Flow~flow.pd(phi1,phi2,phi3,phi4,phi5,time),

fixed=phi1+phi2+phi3+phi4+phi5~type,

random=pdBlocked(list(phi1~1, pdSymm(phi2+phi3+phi4+phi5~1))),

start=c(phi1=2.86,-0.28,phi2=2.92,-1.03,phi3=0.03,0.69,phi4=
-1.76,-0.3,phi5=4.15,-1.51),

data=td,method="ML")
```

One can fit the reduced variance component model by replacing the “random” statement with “random = pdBlocked(list(pdDiag(phi1 + phi3 + phi4 ~ 1), pdSymm(phi2 + phi5 ~ 1)))”, and fit the final model by additionally replacing the “fixed” statement with “fixed = list(phi1 + phi4 ~ 1, phi2 + phi3 + phi5 ~ type)”.

To test whether coronary flow during reperfusion recovered to the pre-ischemic level in WT and KO hearts, the Wald test statistic for WT mice was

$$T = (\hat{\beta}_{11} - \hat{\beta}_{21}) \sqrt{\text{var}(\hat{\beta}_{11} - \hat{\beta}_{21})} = -0.56 \text{ with a } p\text{-value at } 0.57, \text{ indicating that the pre-ischemic coronary flow and the stabilized coronary flow during reperfusion were not significantly different. In the other word, the coronary flow during reperfusion recovered to pre-ischemic level in WT hearts. The Wald test statistic for KO mice was}$$

$$T = (\hat{\beta}_{11} - \hat{\beta}_{21} - \hat{\beta}_{22}) \sqrt{\text{var}(\hat{\beta}_{11} - \hat{\beta}_{21} - \hat{\beta}_{22})} = 2.34 \text{ with a } p\text{-value at } 0.02, \text{ indicating that the pre-ischemic coronary flow for KO mice was significantly higher than the stabilized coronary flow during reperfusion at significant level } 0.05.$$

4.3. Analyses of LVDP

The change point nonlinear mixed-effects model (8) was applied to fit LVDP during-reperfusion observations, and the estimated parameters and their 95% confidence intervals are given in Table 3 under the column “Full model”. We fitted a reduced model excluding some non-significant variance components, however, the LRT for comparing the reduced model and the full model indicated that the two models were significantly different (p -value = 0.011). Therefore, we retained all variance components in the model. Notice that there were several insignificant fixed-effects parameters, we fitted a model without β_{12} and β_{22} ,

and the LRT indicated that this model was not significantly different from the full model (p -value = 0.411). The results for this model are reported in Table 3 under the column title “Final model”. Based on the final model, the predicted subject-level LVDP and its pointwise 95% CI for each individual are presented in Fig. 4A, and the fitted group-level LVDP profile and their pointwise 95% CI for each group were shown in Fig. 4B. Based on the estimation of the parameters (Table 3) and fitted values (Fig. 4), we concluded that: (i) the LVDP before onset for WT mice and KO mice were not significantly different, and the between-subject variation was relative small as σ_1 was relatively small; (ii) the change points for WT mice and KO mice were not significantly different, however, the between-subject variation was relative large as σ_2 was relatively large; (iii) the stabilized LVDP for WT mice was significantly larger than that for KO mice because β_{32} was significantly less than zero; (iv) the increasing rate constant for WT mice was slightly larger than that for KO mice as β_{42} was slightly less than zero, although not significantly (p -value = 0.157). The R-code for analyzing LVDP is shown as follows:

```
t.pd<-function(phi1,phi2,phi3,phi4,time){phi1*(time<= phi2)+(phi1+phi3*(1-exp(-exp(phi4)*(time-phi2))))*(time>= phi2)}
lvdp.full.nlm<-nlme(DEVELOPED~t.pd(phi1,phi2,phi3, phi4,time),
fixed=phi1+phi2+phi3+phi4~type, random=phi1+phi2+phi3+phi4~1,
start=c(phi1=n1start[1],0,phi2=n1start[2],0,phi3=n1start[3],0,phi4=n1start[4],0),
data=td,method="ML")
```

The final model can be obtained with replacing the “fixed” statement by “fixed=list(phi1+phi2~1,phi3+phi4~type)”. The detailed R-code and data sets can be obtained from the corresponding author.

5. Simulation study

In this paper, we applied the proposed models to analyzing an experimental data with a small sample size: 7 WT mice and 6 GSTP-KO mice, where there were 80 observations for each mouse. To examine the performance of the proposed models when the sample size is small or moderate, we carried out simulations under two scenarios: one scenario has a small sample size (say 7 WT mice and 6 GSTP-KO mice), and the other scenario has a moderate sample size (50 WT mice and 50 GSTP-KO mice). Under each scenario and for each cardiac function variable, we chose the final model in Section 4 as the underlying model to generate 1000 simulated data. Each simulated data was generated as follows: we first generated the random effect b_i from $N(0, D)$, where D , the covariance of b_i , was specified by the variance-covariance component parameters in the final model; then we generated the subject-level parameters ϕ_{ik} as $\beta_{k1} + \beta_{k2} type_i + b_{ik}$, and subsequently, we generated the subject-level response profile as $f(\phi_i, t)$; the observations were generated by the subject-level response profile plus random sample from $N(0, \sigma^2)$. We fitted each simulated data set to the final model, the summarized statistics for the 1000 estimated parameters (e.g., mean, variance, and the mean squares for errors (MSE) under each scenario) were, respectively, reported in Tables 4–6 for heart rate, coronary flow, and LVDP. The MSE for each parameter was calculated as the summation of the variance and the squared difference between the sample

mean and the underlying parameter. Based on the simulation results, we conclude that (1) the mean based on the 1000 simulated data under both scenarios are close to the underlying values, indicating that overall the estimates were not biased; (2) as the sample size goes from small to moderate, the variance and MSE dramatically decreased, indicating that the power of test statistics would increase as the sample size goes large.

6. Conclusion and discussion

In this study, we applied the mixed-effects models to the repeated measurements of cardiac function variables including heart rate, coronary flow, and left ventricle developed pressure in the isolated, Langendorff-perfused hearts of male glutathione s-transferase (GSTP1/P2) deficient (KO) mice [15] and WT (C57BL/6) mice. These particular mice were used because GSTP is a known antioxidant enzyme but its role as well as other GSTs in cardioprotection is unclear [16]. For this, we developed piecewise nonlinear mixed-effects models and change point model to describe the different cardiac function variables during the entire experiment. We examined how cardiac function variables are altered by ischemia/reperfusion challenge in KO mice and WT mice using nonlinear mixed-effects model and change point model. The Wald test and Akaike Information Criterion (AIC) are applied to obtain a parsimonious model for each cardiac function variable. The goodness of fit is examined, and the proposed models fit the experimental data very well. Moreover, nonlinear mixed-effects models provide not only interpretable models for the experimental data but also reliable predictions for the response variables in our experimental data. However, the models we developed in this paper are empirical models. In the future, some mechanism-based models may be worth investigating. Particularly, the model developed for coronary flow included four parameters capturing the bell-shaped response profiles. Some simplified mechanism-based model with less number of parameters will be a great interest. In this paper, we applied the models to the experimental data which included only 7 WT mice and 6 GSTP KO mice. When the sample size is small, the variances of the estimated parameters are usually large, and the power of the tests will be low. How to carry out power and sample size analyses is our great interest to work on. However, it is beyond the scope of this paper. Nevertheless, we have demonstrated that nonlinear mixed-effects models can be applied to the assessment of the cardiac function dynamics and the assessment of the treatment effect on the cardiac function. This work provides evidence of a new application for mixed-effects models in physiological and pharmacological studies of the isolated heart.

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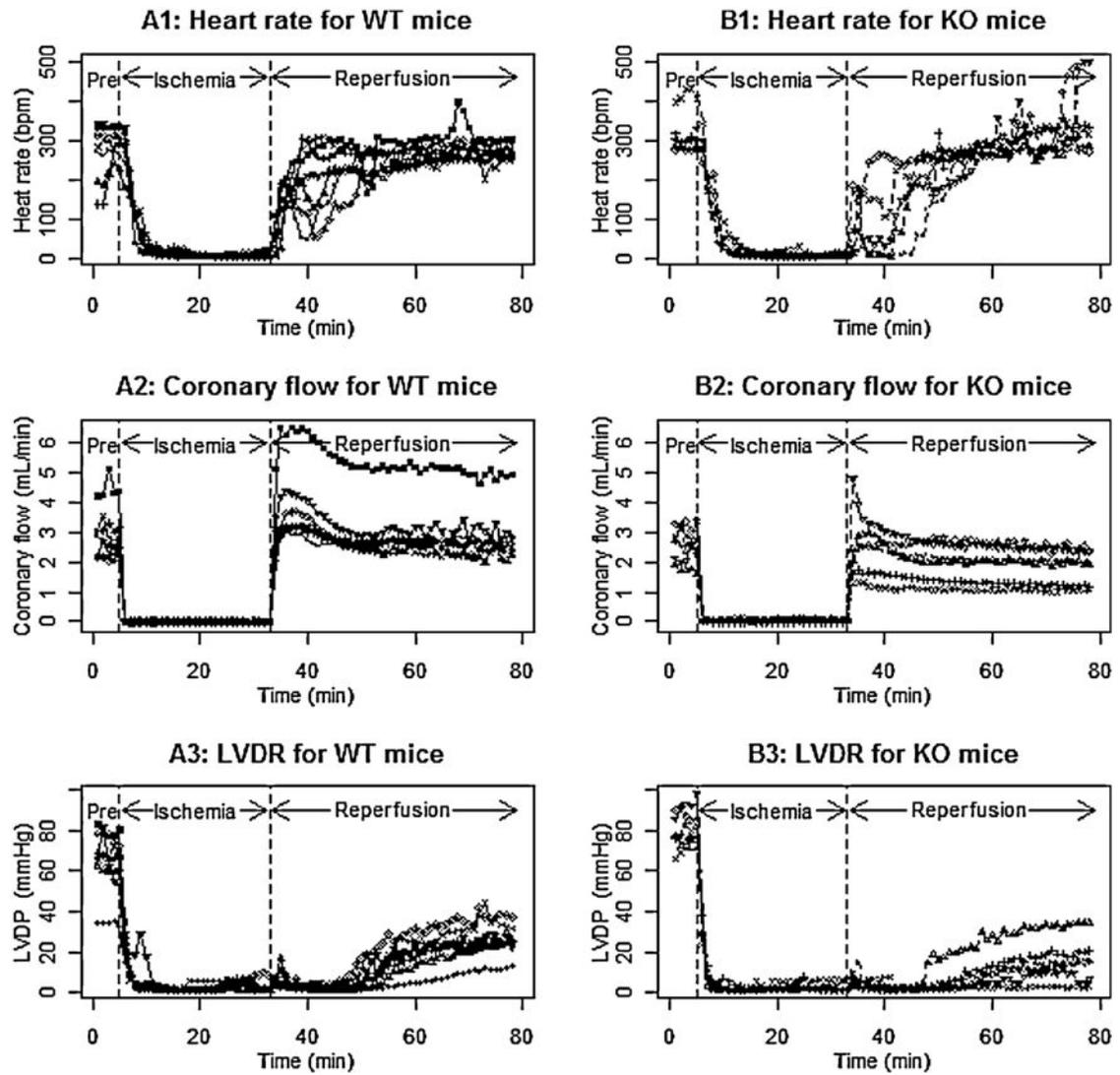


Fig. 1. Individual profiles for heart rate (Panels A1 and B1), coronary flow (Panels A2 and B2), and left ventricle developed pressure (LVDP) (Panels A3 and B3) in WT mice and KO mice.

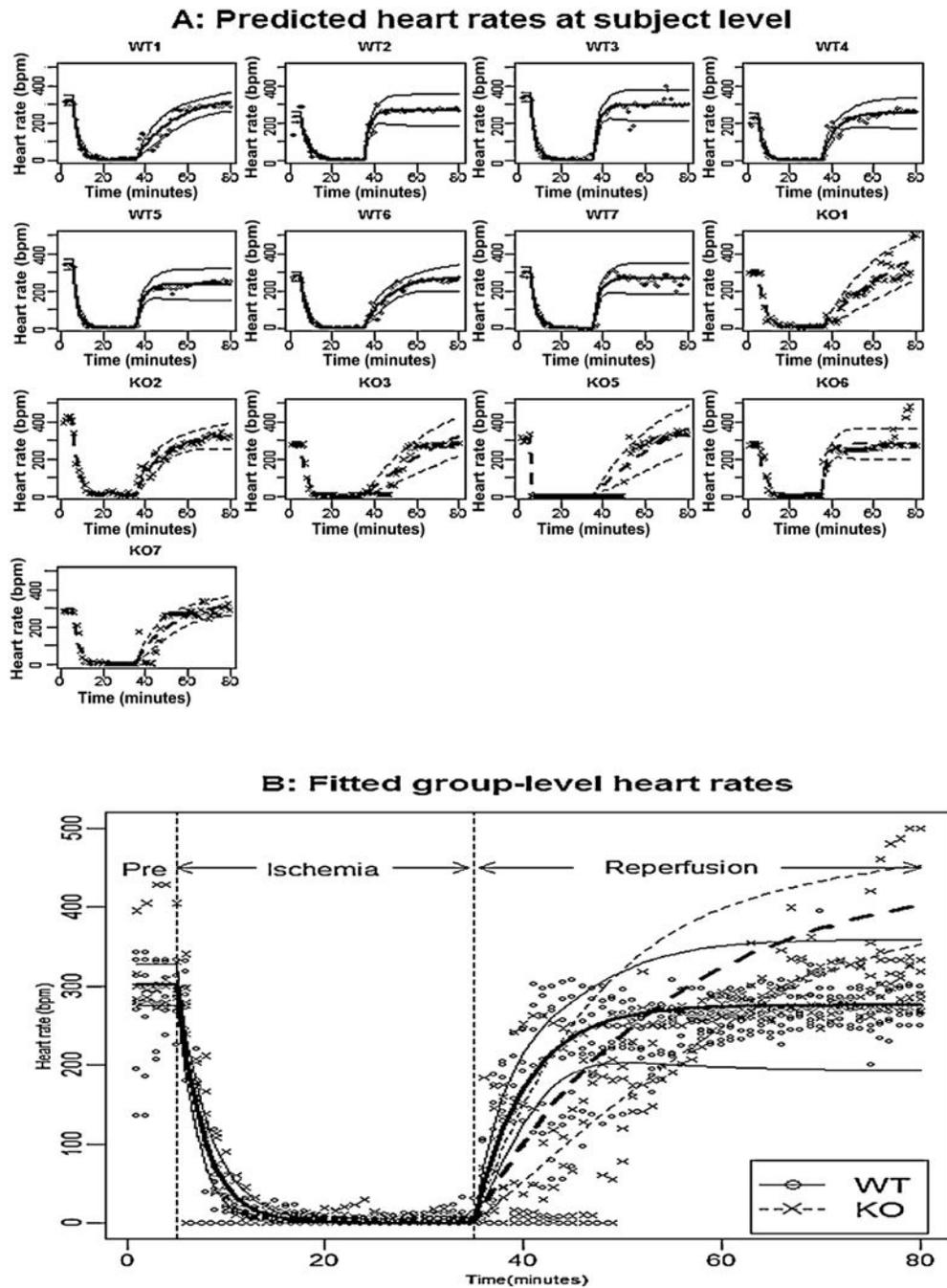


Fig. 2. Panel A shows the observed heart rate for each mouse, the fitted subject-level heart rates, and their pointwise 95% confidence intervals; Panel B shows the observed heart rates for all mice, the fitted group-level heart rates, and their pointwise 95% confidence intervals for WT mice (solid line) and KO mice (dashed line).

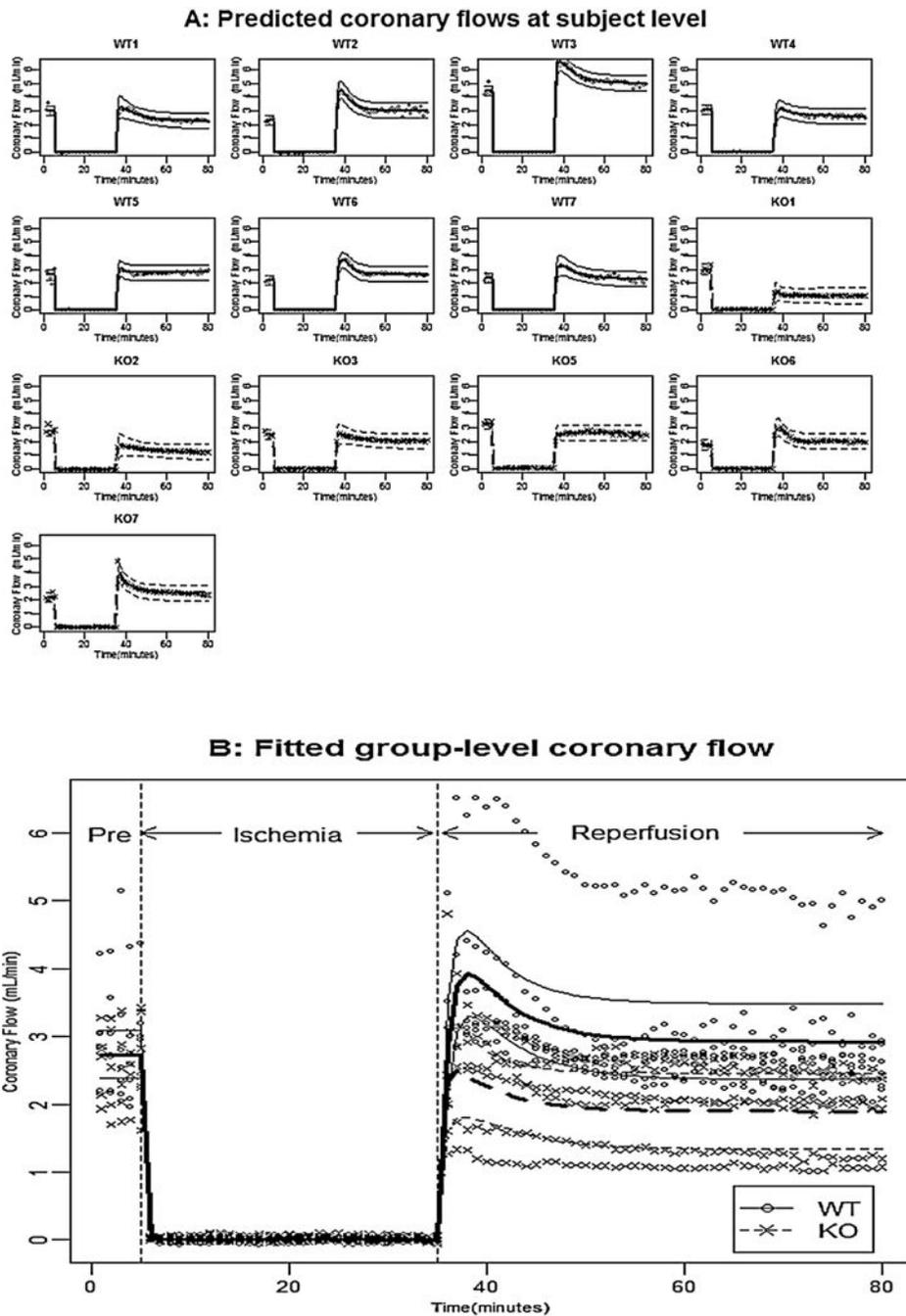
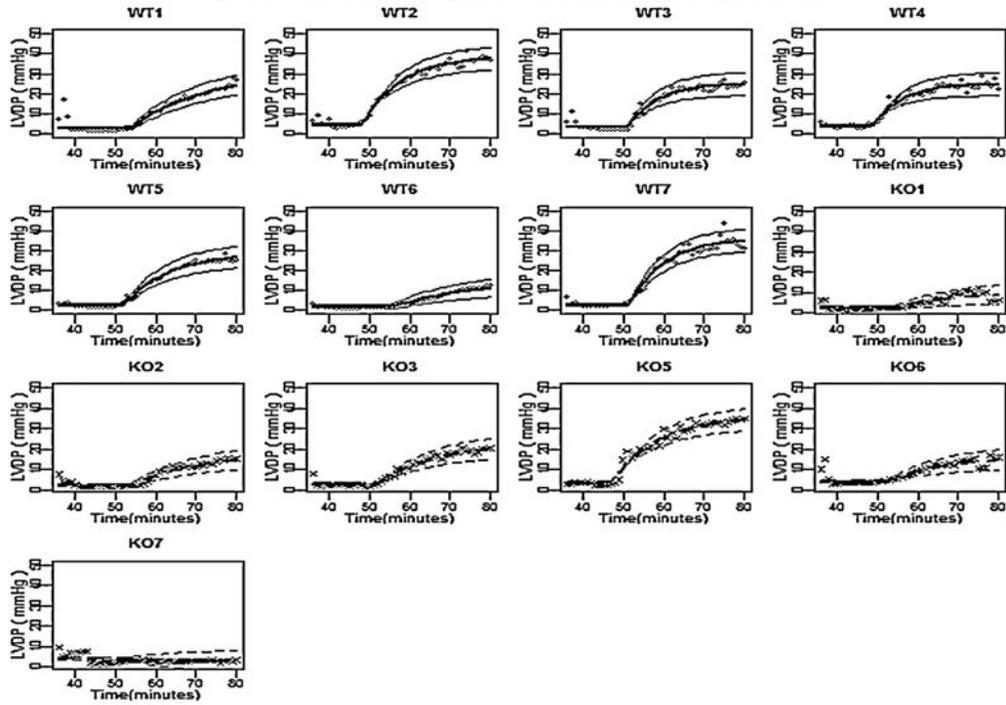


Fig. 3. Panel A shows the observed coronary flow for each mouse, the fitted subject-level coronary flow, and their pointwise 95% confidence intervals; Panel B shows the observed coronary flow for all mice, the fitted group-level coronary flow, and their pointwise 95% confidence intervals for WT mice (solid line) and KO mice (dashed line).

A: Predicted LVDPs at subject level



B: Fitted group-level LVDPs

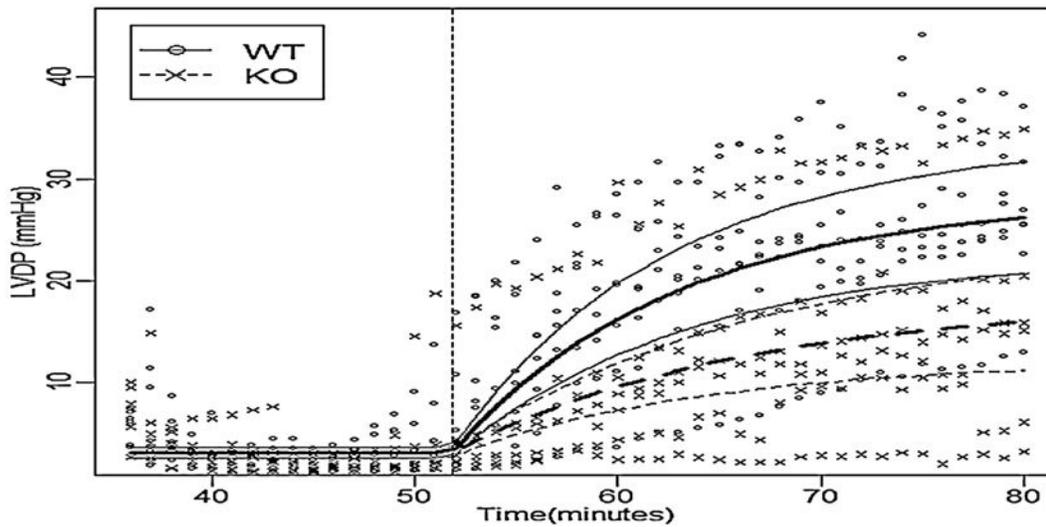


Fig. 4. Panel A shows the observed LVDP for each mouse, the fitted subject-level LVDP, and their pointwise 95% confidence intervals; Panel B shows the observed LVDP for all mice, the fitted group-level LVDP, and their pointwise 95% confidence intervals for WT mice (solid line) and KO mice (dashed line).

Table 1

Estimates of the parameters (Est.) and their 95% confidence intervals (95% CI) for modeling heart rate resulted from the full model, reduced model, and final model.

Parameter	Full model		Reduced variance component model		Final model	
	Est.	(95% CI)	Est.	(95% CI)	Est.	(95% CI)
β_{11}	289.72	(254.73, 324.71)	289.73	(254.77, 324.69)	301.94	(275.36, 328.51)
β_{12}	26.45	(-25.07, 77.97)	26.44	(-25.03, 77.91)		
β_{21}	-1.11	(-1.45, -0.77)	-1.11	(-1.45, -0.77)	-1.01	(-1.26, -0.75)
β_{22}	0.22	(-0.28, 0.72)	0.22	(-0.29, 0.72)		
β_{31}	276.54	(193.43, 359.66)	276.54	(193.44, 359.65)	276.54	(193.36, 359.73)
β_{32}	172.09	(40.59, 303.59)	172.10	(40.62, 303.58)	172.05	(40.44, 303.65)
β_{41}	-1.65	(-2.34, -0.96)	-1.65	(-2.34, -0.96)	-1.65	(-2.34, -0.96)
β_{42}	-1.32	(-2.34, -0.30)	-1.32	(-2.34, -0.30)	-1.32	(-2.34, -0.3)
σ_1	44.92	(29.41, 68.63)	44.88	(29.37, 68.57)	46.64	(30.61, 71.06)
σ_2	0.43	(0.27, 0.71)	0.44	(0.27, 0.72)	0.44	(0.27, 0.72)
σ_3	111.61	(63.25, 196.93)	111.59	(61.42, 202.70)	111.70	(59.88, 208.38)
σ_4	0.92	(0.64, 1.33)	0.92	(0.60, 1.42)	0.92	(0.56, 1.5)
ρ_{12}	0.02	(-0.22, 0.26)				
ρ_{34}	-0.77	(-0.91, -0.45)	-0.77	(-0.92, -0.42)	-0.77	(-0.93, -0.39)
σ	33.40	(31.95, 34.92)	33.40	(31.95, 34.92)	33.41	(31.95, 34.93)
AIC	10,450.04	10,448.04	10,445.73			

Table 2

Estimates of the parameters (Est.) and their 95% confidence intervals (95% CI) for modeling coronary flow resulted from the full model, reduced model, and final model.

Parameter	Full model		Reduced variance component model		Final model	
	Est.	(95% CI)	Est.	(95% CI)	Est.	(95% CI)
β_{11}	2.86	(2.37, 3.35)	2.86	(2.37, 3.35)	2.73	(2.38, 3.10)
β_{12}	-0.28	(-1.00, 0.44)	-0.28	(-0.99, 0.44)		
β_{21}	2.92	(2.34, 3.51)	2.92	(2.35, 3.49)	2.92	(2.35, 3.49)
β_{22}	-1.03	(-1.9, -0.17)	-1.02	(-1.86, -0.19)	-1.02	(-1.86, -0.18)
β_{31}	-0.10	(-0.37, 0.33)	0.01	(-0.52, 0.53)	-0.01	(-0.47, -1.50)
β_{32}	0.72	(-0.11, 0.94)	0.81	(-0.03, 1.64)	0.71	(-0.05, 1.48)
β_{41}	-1.89	(-2.20, -1.20)	-1.70	(-2.12, -1.27)	-1.77	(-2.08, -1.47)
β_{42}	-0.19	(-1.14, 0.40)	-0.00	(-0.66, 0.67)		
β_{51}	4.12	(3.18, 5.17)	4.18	(3.30, 5.06)	4.18	(3.29, 5.06)
β_{52}	-1.52	(-2.92, -0.01)	-1.56	(-2.85, -0.27)	-1.50	(-2.80, -0.20)
σ_1	0.66	(0.44, 0.98)	0.65	(0.44, 0.97)	0.67	(0.44, 0.96)
σ_2	0.77	(0.51, 1.15)	0.69	(0.45, 1.05)	0.63	(0.51, 1.11)
σ_3	0.70	(0.46, 1.07)	0.52	(0.34, 0.80)	0.47	(0.29, 0.55)
σ_4	0.54	(0.35, 0.83)	0.77	(0.52, 1.14)	0.77	(0.26, 0.60)
σ_5	1.17	(0.78, 1.77)	1.17	(0.79, 1.74)	1.18	(0.83, 1.89)
ρ_{23}	0.19	(-0.35, 0.63)				
ρ_{24}	-0.17	(-0.59, 0.32)				
ρ_{25}	0.85	(0.59, 0.95)	0.85	(0.60, 0.95)	0.84	(0.57, 0.95)
ρ_{34}	-0.44	(-0.80, 0.16)				
ρ_{35}	0.24	(-0.29, 0.66)				
ρ_{45}	-0.02	(-0.48, 0.44)				
σ	0.10	(0.10, 0.11)	0.10	(0.10, 0.11)	0.10	(0.10, 0.11)
AIC	-1440.10		-1460.48		-1461.07	

Table 3

Estimates of the parameters (Est.) and their 95% confidence intervals (95% CI) for modeling left ventricle developed pressure (LVDP) during reperfusion based on full model and final model.

Parameter	Full model		Final model	
	Est.	(95% CI)	Est.	(95% CI)
β_{11}	3.22	(2.53, 3.90)	3.14	(2.64, 3.64)
β_{12}	-0.15	(-1.15, 0.86)		
β_{21}	51.60	(49.30, 53.89)	51.80	(50.06, 53.53)
β_{22}	0.50	(-3.05, 4.04)		
β_{31}	25.26	(18.75, 31.77)	24.94	(19.05, 30.82)
β_{32}	-10.50	(-20.13, -0.88)	-9.96	(-17.17, -2.75)
β_{41}	-2.38	(-2.61, -2.16)	-2.40	(-2.61, -2.19)
β_{42}	-0.30	(-0.71, 0.11)	-0.27	(-0.63, 0.09)
σ_1	0.80	(0.48, 1.33)	0.79	(0.47, 1.33)
σ_2	3.02	(1.91, 4.78)	3.02	(1.96, 4.66)
σ_3	8.58	(5.74, 12.82)	8.74	(5.87, 13.02)
σ_4	0.24	(0.08, 0.71)	0.23	(0.08, 0.67)
ρ_{12}	-0.62	(-0.92, 0.18)	-0.66	(-0.93, 0.10)
ρ_{13}	0.18	(-0.46, 0.70)	0.18	(-0.44, 0.68)
ρ_{14}	0.48	(-0.52, 0.92)	0.47	(-0.52, 0.92)
ρ_{23}	-0.70	(-0.93, -0.11)	-0.68	(-0.92, -0.11)
ρ_{24}	-0.60	(-0.98, 0.74)	-0.67	(-0.97, 0.41)
ρ_{34}	0.08	(-0.89, 0.92)	0.13	(-0.70, 0.81)
σ	1.84	(1.74, 1.96)	1.85	(1.74, 1.96)
AIC	2540.96		2538.73	

Table 4

Simulation results based on the final model for heart rate and 1000 simulated data sets.

Parameter	True value	$n_{WT} = 7$ & $n_{GSTP} = 6$			$n_{WT} = 50$ & $n_{GSTP} = 50$		
		Mean	Variance	MSE	Mean	Variance	MSE
β_{11}	301.9	302.2	175.4	175.5	302.2	24.65	24.76
β_{21}	-1.01	-1.02	0.02	0.02	-1.02	<0.01	<0.01
β_{31}	276.5	275.3	1674.8	1676.4	275.7	251.5	252.3
β_{32}	172.0	166.2	4456.5	4490.5	170.7	542.3	544.0
β_{41}	-1.65	-1.67	0.11	0.11	-1.67	0.02	0.02
β_{42}	-1.32	-1.29	0.25	0.25	-1.29	0.03	0.04

Simulation results based on the final model for coronary flow and 1000 simulated data sets.

Table 5

Parameter	True value	$n_{WT} = 7$ & $n_{GSTP} = 6$			$n_{WT} = 50$ & $n_{GSTP} = 50$		
		Mean	Variance	MSE	Mean	Variance	MSE
β_{11}	2.73	2.73	0.03	0.03	2.73	0.01	0.01
β_{21}	2.92	2.91	0.08	0.08	2.92	0.01	0.01
β_{32}	-1.02	-1.01	0.18	0.18	-1.02	0.01	0.01
β_{31}	0.01	0.06	0.02	0.03	0.01	0.01	0.01
β_{32}	0.71	0.64	0.11	0.12	0.71	0.01	0.01
β_{41}	-1.77	-1.75	0.02	0.04	-1.77	0.01	0.01
β_{51}	4.18	4.03	0.19	0.21	4.18	0.04	0.04
β_{52}	-1.50	-1.47	0.42	0.43	-1.50	0.04	0.04

Table 6

Simulation results based on the final model for LYDP and 1000 simulated data sets.

Parameter	True value	$n_{WT} = 7$ & $n_{GTSP} = 6$			$n_{WT} = 50$ & $n_{GTSP} = 50$		
		Mean	Variance	MSE	Mean	Variance	MSE
β_{11}	3.14	3.20	0.05	0.05	3.14	0.01	0.01
β_{21}	51.80	51.22	0.44	0.76	51.83	0.10	0.10
β_{31}	24.94	24.89	6.60	6.60	24.85	1.13	1.14
β_{32}	-9.96	-10.43	12.69	12.91	-9.96	1.41	1.41
β_{41}	-2.40	-2.47	0.01	0.02	-2.40	<0.01	<0.01
β_{42}	-0.27	-0.26	0.02	0.02	-0.27	<0.01	<0.01